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(54) 【発明の名称】末端分枝高分子リンカーおよびそれを含む高分子複合体

(57)【要約】

多数のローディングが可能な末端分枝高分子プロドラッグプラットフォームを開示する。本発明の好ましい態様では、プロドラッグブラットフォームは活性物質を保持している各分枝がベンジル脱離反応を受けた後に多数の親化合物を放出する。プロドラッグの製造方法および哺乳類の治療におけるその使用方法もまた開示する。1つの好ましい態様では、式(I)などの高分子複合体を提供する。

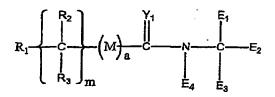
【特許請求の範囲】

【請求項1】

: [法

【化1】。

(I)



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(式中、

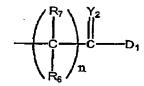
R₄は高分子残基であり;

Yı はO、SまたはNR, であり;

MはO、SまたはNR、であり;

E, は

【化2】。

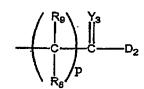


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であり;

E_4は独立に、H、EIまたは

[化3]



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であり;

(a)はOまたは1であり;

(m)はOまたは正の整数であり;

(n)および(p)は独立に、0または正の整数であり;

Y₂₋₃は独立に、O、SまたはNR₁₀であり;

 R_{2-10} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{1-6} 40 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} へテロアルキル、置換 C_{1-6} へテロアルキル、 C_{1-6} へテロアルキシ、フェノキシおよび C_{1-6} へテロアルコキシからなる群から選択され;

D,およびD,は独立に、OH、

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【化4】

(井田

(v)および(t)は独立に、0または約6までの正の整数であり;

JはNR, または

【化5】

~h & &

であり;

L、およびL、は独立に選択された二官能性リンカーであり;

Y₄₋₇は独立に、O、SおよびNR₁₄からなる群から選択され;

 R_{11-14} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{1-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} 40 ヘテロアルキル、置換 C_{1-6} ヘテロアルキル、 C_{1-6} アルコキシ、フェノキシおよび C_{1-6} ヘテロアルコキシからなる群から選択され;

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり;

B,およびB,は独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含有成分の残基からなる群から選択される)

または末端分枝基である}

で表される化合物。

【請求項2】

R₄が水素、NH₂、OH、CO₂H、C₁₋₆基および

【化6】

$$E_{2} = C = N - C - (M)_{a} = \begin{pmatrix} R_{2} \\ C \\ R_{3} \end{pmatrix}_{m}$$

からなる群から選択されるキャッピング基Aをさらに含んでなる、請求項1に記載の化合物。

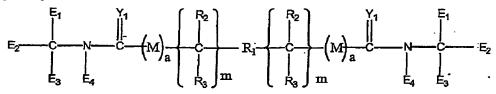
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【請求項3】

式:

【化7】

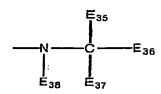


で表される、請求項2に記載の化合物。

【請求項4】

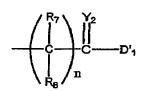
上記末端分枝基が式:

【化8】



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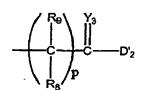
武中、 E,,は 【化9】



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であり;

E₃₆₋₃₈は独立に、H、E₃₅または 【化10】



であり;

(n)および(p)は独立に、0または正の整数であり;

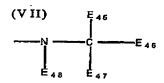
Y2-3は独立に、O、SまたはNR10であり;

 R_{6-10} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{1-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} へテロアルキル、置換 C_{1-6} へテロアルキル、 C_{1-6} アルコキシ、フェノキシおよび C_{1-6} へテロアルコキシからなる群から選択され;

D',およびD',は独立に、OH、

【化11】

または 【化12】



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(土)

(v)および(t)は独立に、0または約6までの正の整数であり;

L、およびL、は独立に選択された二官能性リンカーであり;

 Y_{4-7} は独立に、O、Sおよび NR_{4} からなる群から選択され;

 R_{1-14} は独立に、水素、 G_{-6} アルキル、 G_{3-12} 分枝鎖アルキル、 G_{3-8} シクロアルキル、 G_{-6} 置換アルキル、 G_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 G_{1-6} ヘテロアルキル、置換 G_{1-6} ヘテロアルキル、 G_{1-6} マアルコキシからなる群から選択され;

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり;

 B_1 および B_2 は独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含有成分の残基からなる群から選択され;

E, は

【化13】

$$- \left(\begin{matrix} \begin{matrix} R_7 \\ \end{matrix} \end{matrix} \right) \begin{matrix} Y_2 \\ C \\ \end{matrix} \begin{matrix} C \end{matrix} \begin{matrix} D"_1 \end{matrix}$$

であり;

E₄₆₋₄₈は独立に、H、E₄₅または 【化14】

$$- \left(\begin{array}{c} R_{\theta} \\ C \\ R_{\theta} \end{array} \right) \stackrel{Y_3}{\underset{C}{\longrightarrow}} D"_2$$

(式中、

D",およびD"。は独立に、OH、

【化15】

または 【化16】

である)

である}

である]

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で表される、請求項1に記載の化合物。

【請求項5】

Y,が0である、請求項3に記載の化合物。

【請求項6】

R,がポリアルキレンオキシド残基を含んでなる、請求項1に記載の化合物。 【請求項7】

R,がポリエチレングリコール残基を含んでなる、請求項6に記載の化合物。 【請求項8】

R₁がポリエチレングリコール残基を含んでなる、請求項3に記載の化合物。 【請求項9】

R が

-C(=Y₆)-(CH₂)₆-0-(CH₂CH₃O)₃-A₃

 $-C(=Y_6)-Y_7-(CH_2)_6-0-(CH_2CH_2O)_*-A$

 $-C(=Y_6)-NR_2$, $-(CH_2)_f$, $-O-(CH, CH, O)_r$, $-A_1$

 $-(CR_{2}, R_{2}, R_{3}, R_{4}, R_{5}, R_{5$

-NR, , -(CH,), -O-(CH, CH, O), -A,

 $-C(=Y_6)-(CH_2)_f-0-(CH_3CH_3O)_x-(CH_3)_f-C(=Y_6)-(CH_3CH_3O)_x$

 $-C(=Y_6)-Y_7-(CH_2)_4-O-(CH_2CH_2O)_4-(CH_3)_4-Y_7-C(=Y_6)-X_7$

 $-C(=Y_6)-NR_2$, $-(CH_1)_6-O-(CH_1,CH_2,O)_2$, $-(CH_1)_6-NR_2$, $-C(=Y_6)-C(-Y_6)_6$

 $-(CR_{24}R_{25})_{e}-0-(CH_{20})_{f}-0-(CH_{20}CH_{20})_{x}-(CH_{20})_{f}-0-(CR_{24}R_{25})_{e} \Rightarrow \downarrow V$

 $-NR_{2,3}$ $-(CH_{2})_{f}$ $-0-(CH_{2}CH_{2}O)_{x}$ $-(CH_{2})_{f}$ $-NR_{2,3}$ -

(式中、 > お t バ> は

Y₆およびY₇は独立に、O、SまたはNR₂₃であり;

xは重合度であり;

 R_{23} 、 R_{24} および R_{25} は独立に、H、 G_{-6} アルキル、 G_{3-12} 分枝鎖アルキル、 G_{3-8} シクロアルキル、 G_{-6} 置換アルキル、 G_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 G_{-6} ヘテロアルキル、置換 G_{-6} ヘテロアルキル、 G_{-6} アルコキシ、フェノキシおよび G_{-6} ヘテロアルコキシからなる群から選択され;

eおよびfは独立に、O、1、または2であり;かつ

Aはキャッピング基である)

からなる群から選択される、請求項6に記載の化合物。

【請求項10】

R,が-O-(CH, CH, O), を含んでなり、かつxは重量平均分子量が少なくとも約20,000であるような正の整数である、請求項9に記載の化合物。

【請求項11】

R,の重量平均分子量が約20,000~約100,000である、請求項3に記載の化合物。

【請求項12】

R の重量平均分子量が約25,000~約60,000である、請求項3に記載の化合物。

【請求項13】

式

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【化17】

で表される、請求項3に記載の化合物。

【請求項14】

りが

【化18】

である、請求項13に記載の化合物。

【請求項15】

D₁が

【化19】

である、請求項13に記載の化合物。

【請求項16】

L, が(CH, CH, O), である、請求項1に記載の化合物。

【請求項17】

 L_{2} ± 0.00 \pm 、-(CH,), -NH-C(O)(CH,), NH-および-CH, C(O)NHCH(CH, CH(CH,),)-からなる群から選択され 50

る、請求項1に記載の化合物。

【請求項18】 【化20】

および 【化21】

{式中、 R, はPEG残基であり、かつDは

【化22】

および 【化23】

(式中、

Bはアミンまたはヒドロキシル基含有薬物の残基である)

からなる群から選択される}

からなる群から選択される、請求項1に記載の化合物。

【請求項19】

Bがダウノルビシン、ドキソルビシン; p-アミノアニリンマスタード、メルファラン、Ara-C(シトシンアラビノシド)、ロイシン-Ara-C、およびゲムシタビンからなる群のメンバーの残基である、請求項18に記載の化合物。

【請求項20】

治療が必要な哺乳類に、有効量の請求項1に記載の化合物(式中、D₁は生物学上活性な成分の残基である)を投与することを含んでなる、治療方法。

【請求項21】

治療が必要な哺乳類に有効量の請求項18に記載の化合物を投与することを含んでなる、 40治療方法。

【請求項22】

Arが式:

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(式中、

 R_{11} および R_{16-20} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアル 10 キル、 C_{1-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} ヘテロアルキル、「10 投 C_{1-6} へテロアルキン、フェノキシおよび C_{1-6} へテロアルコキシからなる群から選択される)

で表される、請求項1に記載の化合物。

【請求項23】

R1.およびR18-20が各々、HまたはCH,である、請求項22に記載の化合物。

【請求項24】

高分子複合体の製造方法であって、

式(VIII):

【化25】

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、中、

(v)および(t)は独立に、0または約6までの正の整数であり;

JはNR, または

【化26】

であり;

L,およびL,は独立に選択された二官能性リンカーであり;

Y₄₋,は独立に、O、SおよびNR₁,からなる群から選択され;

 R_{1-17} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{4-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} へテロアルキル、置換 C_{1-6} へテロアルキル、 C_{1-6} マルコキシ、フェノキシおよび C_{1-6} ヘテロアルコキシからなる群から選択され;

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分

であり;かつ

B', はヒドロキシルまたはアミン基含有成分の残基である)で表される化合物と、式(IX):

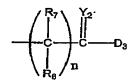
【化27】

$$R_{1} = \left\{ \begin{array}{c} R_{2} \\ C \\ R_{3} \end{array} \right\}_{m} \left(M \right)_{a} C \left(M \right)_{a} \left(K_{3} \right)_{a} \left(K_{3$$

(式中、

足は

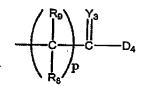
【化28】



であり;

E₆₋₈は独立に、H、E₅または

【化29】



であり:

D,およびD,は独立に、OH、保護されていないアミンまたはヒドロキシルと反応しうる脱離 30基、または末端分枝基であり;

R は高分子残基であり;

MはO、SまたはNR、であり;

(a)は0または1であり;

(m)は0または正の整数であり;

(n)および(p)は独立に、Oまたは正の整数であり;

Y₂₋₃は独立に、O、SまたはNR₄であり;かつ

 R_{2-10} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{4-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{4-6} ペテロアルキル、置換 C_{4-6} ペテロアルキル、 C_{4-6} アルコキシからなる群から選択される)

で表される化合物とを、高分子複合体を生成させるのに十分な条件下で反応させることを含んでなる、上記方法。

【発明の詳細な説明】

【技術分野】

[0001]

本発明は生物活性材料の長時間作用性複合体の作製に有用である新しいタイプの末端活性 化高分子材料に関する。特に、本発明は治療的ペイロードの高い高分子系複合体およびそ の製造方法に関する。

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【背景技術】

[0002]

長年にわたり、生物学上有効な材料を哺乳類に投与するいくつかの方法が提案されてきた。多くの薬剤が水溶性塩として入手可能であり、比較的容易に医薬製剤に配合することができる。所望の薬剤が液体に不溶性である場合、またはin vivoで急速に分解される場合には問題が起こる。特に、アルカロイドは難溶である場合が多い。

[0003]

薬剤を可溶性にする1つの方法がそれらを可溶性プロドラッグの一部として含める方法である。プロドラッグは投与した際にin vivoにおいて最終的に親化合物を遊離する生物学上活性な親化合物の化学誘導体を含む。プロドラッグは当業者によるin vivoにおける薬剤作用の発現および/または持続時間の改変を可能とし、体内での薬物の輸送、分配または溶解性を改変しうるものである。さらに、プロドラッグ製剤は毒性を減弱することも多く、あるいはまた医薬製剤を投与する場合に遭遇する問題を克服もする。プロドラッグの典型例としては、有機リン酸塩またはアルコールもしくはチオアルコールのエステルが挙げられる。Remington's Pharmaceutical Sciences, 16th Ed., A. Osol, Ed. (1980)を参照。なお、その開示は参照により本明細書に組み入れる。

[0004]

プロドラッグは親化合物または活性化合物の生物学上不活性または実質的に不活性な形態である場合が多い。活性薬物の放出速度、すなわち、加水分解速度はいくつかの要因によって、特に、親薬物と改変剤とをつなぐ結合タイプにより影響を受ける。親化合物の十分 20 な量の加水分解が起こる前に腎臓または細網内皮系などにより排出されるプロドラッグを製造することがないよう留意しなければならない。

[0005]

高分子をプロドラッグ系の一部として組み込むことで薬物の循環寿命が長くなることが示されている。しかしながら、約10,000ダルトン未満の1種のみまたは2種の高分子各々とアルカロイド化合物などの特定の生物学上活性な物質とを複合体化した場合、特に、幾分耐加水分解性の結合が使用されている場合には、得られた複合体がin vivoで迅速に排出されることが分かっている。実際、かかる複合体は体から極めて迅速に排出されるため、加水分解が起こりやすいエステル結合が使用されている場合でさえも、治療効果に十分な親分子がin vivoにおいて再生されない。

[0006]

カンプトテシンおよび生物学上活性な関連類似体は水溶性に乏しいことが多く、PEGプロドラッグ技術によって恩恵を得る物質の例である。当技術分野でのこれまでのいくつかの研究の概要を以下に示す。

[0007]

Ohya, ら、J. Bioactive and Compatible Polymers Vol. 10 Jan., 1995, 51–66では、エステルをはじめとする種々の結合を介した2置換基の結合により作製されるドキソルビシン-PEG複合体を開示している。しかしながら、使用したPEGの分子量はせいぜい約5,000である。そのため、複合体は十分な結合の加水分解の前に実質的に排出されることから、in vivoにおける恩恵の実現は十分なものではない。

[00008]

米国特許第4,943,579号では、水溶性プロドラッグとして塩形態の特定の単純な20(S)-カンプトテシンアミノ酸エステルを開示している。しかし、参考文献ではアルカロイドを比較的高分子量の高分子に結合させてプロドラッグを作製するための結合の一部としてアミノ酸を使用することについては開示されていない。表2で示された579人の患者に関するデータから明らかなように、加水分解は急速である。そのため、生理学的pHでは注入後に不溶性基剤が迅速に生じ、タンパク質と結合し、治療効果が達成される前に体から迅速に排出される。関連した取り組みは水溶性カンプトテシンナトリウム塩の開発に向けられた。【0009】

残念なことに、カンプトテシンの水溶性ナトリウム塩には依然として臨床応用に対する高 50

い有害性が残されていた(Gottlieb ら, 1970 Cancer Chemother, Rep. 54, 461; Moertel ら, 1972 上記, 56, 95; Gottlieb ら, 1972 上記, 56, 103)。

[0010]

本願出願人によるPCT公報W096/23794では、1当量のヒドロキシル基含有薬物を高分子の各末端に結合したビス-複合体について記載している。このような進展があったものの高分子のペイロードをさらに高める技術が求められている。

[0011]

このように、カンプトテシンおよび関連類似体などの治療成分からなるプロドラッグを作製するさらなる技術を提供する必要性がなお存在している。本発明ではこの必要性に取り組むものである。

【発明の開示】

[0012]

本発明の1つの態様では、式(I):

【化1】

(I) $R_{1} = \left\{ \begin{array}{c} R_{2} \\ C \\ R_{3} \end{array} \right\}_{\mathbf{m}} \left\{ \begin{array}{c} Y_{1} \\ M \\ A \end{array} \right\}_{\mathbf{E}_{4}} \left\{ \begin{array}{c} E_{1} \\ E_{3} \end{array} \right\}_{\mathbf{E}_{2}}$

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[0013]

(式中、

R₁は高分子残基であり;

Y, はO、SまたはNR。であり;

MはO、SまたはNR、であり;

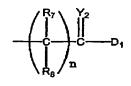
(m)は0または正の整数、好ましくは1または2であり;

(a)は0または1であり;

E, は

【化2】

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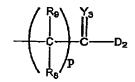
[0014]

であり;

E2-4は独立に、H、E、または

【化3】

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[0015]

であり;

(n)および(p)は独立に、0または正の整数であり;

 Y_{2-3} は独立に、O、SまたはNR。であり;

 R_{2-10} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{1-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} ヘテロアルキル、置換 C_{1-6} ヘテロアルキル、 C_{1-6} へテロアルキンからなる群から選択され;

D,およびD,は独立に、OH、

[化4]

[0016]

または以下に示すさらなる末端分枝基である)

で表される化合物が提供される。

[0017]

式(IV)および(V)中、

(v)および(t)は独立に、0または約6までの正の整数、好ましくは約1であり;

JはNR, または

【化5】

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[0018]

であり;

L,およびL,は独立に選択された二官能性リンカーであり;

Y₄₋,は独立に、O、SおよびNR,,からなる群から選択され;

 R_{11-17} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{1-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} へテロアルキル、置換 C_{3-6} へテロアルキル、 C_{3-6} フェノキシおよび C_{3-6} 50

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ロアルコキシからなる群から選択され;

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり;かつ

 B_1 および B_2 は独立に、脱離基、OH、ヒドロキシルまたはアミン基含有成分の残基からなる群から選択される。

[0019]

本発明の1つの特に好ましい態様では、高分子残基の末端部が次の式(II):

[146]

(II) $E_{2} = \begin{bmatrix} E_{1} & Y_{1} & \vdots & \vdots & \vdots \\ E_{3} & E_{4} & \vdots & \vdots & \vdots \\ E_{3} & E_{4} & \vdots & \vdots & \vdots \end{bmatrix} \begin{bmatrix} R_{2} & \vdots & \vdots & \vdots \\ R_{3} & \vdots & \vdots & \vdots \\ R_{3} & \end{bmatrix} m$

[0020]

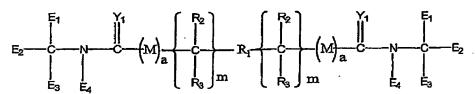
(式中、

全ての置換基および変数はこれまでに定義したとおりである)

で表される成分でさらに置換されている。よって、二官能性化合物は、本明細書において B_{α} または B_{α} とよばれる、2、4またはそれ以上の当量の生物学上活性な薬剤、薬物、または タンパク質が送達されうるように高分子残基 (R_{α}) が α および α 両方の末端結合基を含有す 20 る場合に形成される。かかる二官能性高分子輸送形態の例は次の式(III):

【化7】

(M)



[0021]

(式中、

全ての置換基および変数は上記のとおりである)

のように示される。

[0022]

本発明の目的における「残基」とは、生物学上活性な化合物がプロドラッグ担体部分を結合するための置換反応を受けた後にも残存する生物学上活性な化合物の部分を意味するものとする。

[0023]

本発明の目的における「アルキル」とは、直鎖、分枝鎖、置換(例えば、ハロー、アルコキシー、およびニトロー) G_{-1} アルキル、 G_{3-8} シクロアルキルまたは置換シクロアルキル 40などを包含するものとする。

[0024]

本発明の目的における「置換」とは、官能基または化合物に含まれる1個以上の原子に1個以上の異なる原子を付加するまたはそれと置き換えることを包含するものとする。

[0025]

本発明の目的において、「置換アルキル」とは、カルボキシアルキル、アミノアルキル、ジアルキルアミノ、ヒドロキシアルキルおよびメルカプトアルキルを包含し;「置換シクロアルキル」とは、4-クロロシクロヘキシルなどの基を包含し;「アリール」とは、ナフチルなどの基を包含し;「置換アリール」とは、3-ブロモフェニルなどの基を包含し;「アラルキル」とは、トルイルなどの基を包含し;「ヘテロアルキル」とは、エチルチオフ 50

ェンなどの基を包含し;「置換ヘテロアルキル」とは、3-メトキシ-チオフェンなどの基を包含し;「アルコキシ」とは、メトキシなどの基を包含し;および「フェノキシ」とは、3-ニトロフェノキシなどの基を包含する。ハロ-はフルオロ、クロロ、ヨードおよびブロモを包含するものとする。

[0026]

本発明の目的における「十分な量」とは、当業者によって理解される治療効果を達成する量を意味するものである。

[0027]

本発明の化合物の最も重要な利点の1つはプロドラッグの高分子単位当たりのペイロードがこれまでの技術よりも高いことである。一般的には、高分子がまず加水分解によりトリ 10メチルロック (TML)系プロドラッグ中間体を放出し、次いで得られた中間体または「第2のプロドラッグ」部分がラクトン化を受けて、例えば、生物学上活性な化合物またはさらなるプロドラッグを含んでなる組成物 (composition)のいずれかである成分を再生することが好ましい。よって、本発明の高ペイロード高分子複合体は最大4つまでまたはそれ以上の数の薬物分子を含有しうる独自の送達系である。

[0028]

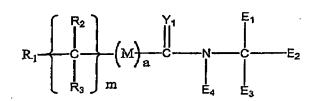
本明細書において記載する化合物および複合体の製造および使用方法も提供される。 【0029】

発明の詳細な説明

A.<u>式(I)</u>

本発明の1つの好ましい実施形態では、式:

【化8】



[0030]

(式中、

(I)

R,は高分子残基であり;

Y, はO、SまたはNR, であり;

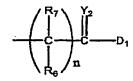
MはO、SまたはNR、であり;

(a)は0または1であり;

(m)は0または正の整数であり:

見は

【化9】



[0031]

であり;

E__4は独立に、H、E、または

【化10】

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$$- \left(\begin{matrix} R_9 \\ C \\ R_0 \end{matrix} \right) \begin{matrix} Y_3 \\ C \\ P \end{matrix} - D_2$$

[0032]

であり;

(n)および(p)は独立に、0または正の整数であり;

Y₂₋₃は独立に、O、SまたはNR₁。であり;

 R_{2-10} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{4-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{4-6} へテロアルキル、 C_{4-6} でルコキシ、フェノキシおよび C_{4-6} でルコキシからなる群から選択され;

D,およびD,は独立に、OH、

【化11】

【0033】 C式中、 JはNR₁,または 【化12】



【0034】 であり; 40

(v)および(t)は独立に、0または約6までの正の整数、好ましくは約1であり; L,およびL,は独立に選択された二官能性リンカーであり;

Y₄₋,は独立に、O、SおよびNR₁,からなる群から選択され;

 R_{1-17} は独立に、水素、 G_{-6} アルキル、 G_{-12} 分枝鎖アルキル、 G_{-8} シクロアルキル、 G_{-6} 置換アルキル、 G_{-6} 置換シクロアルキル、アリール、置換アリール、アラルキル、 G_{-6} へテロアルキル、置換 G_{-6} へテロアルキル、 G_{-6} アルコキシ、フェノキシおよび G_{-6} へテロアルコキシからなる群から選択され;

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり;かつ

 B_1 および B_2 は好ましくは独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含 10 有成分の残基からなる群から選択される)

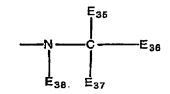
である}

で表される化合物が提供される。

[0035]

もう1つの好ましい実施形態では、D,およびD,は独立に、式(VI) 【化 1 3】

(VI)



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[0036]

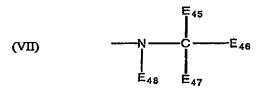
(式中、

 $E_{3,5-3,8}$ は定義内で D_1 および D_2 が以下で定義する D'_1 および D'_2 に変わることを除き、上記の E_{1-4} の定義と同じ基から選択される)

で表される選択された末端分枝基である。この実施形態では、D',およびD',が独立に、OH、式(IV)または(V)で表される成分、または

【化14】

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[0037]

、中、

E45-48 は定義内でD1およびD2がD"1およびD"2に変わり、かつD"1およびD"2が独立に、OH、 40式(IV)または式(V)であることを除き、E1-4の定義と同じ基から選択される) で表される成分でありうる。上記のことからわかるように、末端分枝がその最大限に二官能性高分子R2を受け入れるとすると、最大16当量の薬物が高分子プラットフォームにロード(load)できる。

[0038]

ビス-置換高分子残基が望まれるこの実施形態の態様では、本発明のいくつかの好ましい 高分子輸送系が次の式

【化15】

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(III): $E_{2} = \begin{bmatrix} E_{1} & Y_{1} & E_{1} \\ C & N & C \end{bmatrix} \begin{pmatrix} R_{2} \\ R_{3} \end{pmatrix} m \begin{pmatrix} R_{2} \\ R$

[0039]

、中、

全ての置換基および変数はこれまでに記載したとおりである)

のように示される。

[0040]

本発明のマルチ・ローディング(multi-loading)高分子輸送系は主として本明細書においてR、とよばれる高分子残基に基づくものである。所望により、R、がキャッピング基Aを含有していてもよい。高分子キャッピング基Aとしては、例えば、水素、CO,H、C、6アルキル基、およびビス系を形成する以下に示す式(II)の化合物などの成分が挙げられる:

【化16】

(II) $E_{2} \longrightarrow \begin{bmatrix} E_{1} & Y_{1} \\ C & -X_{1} \end{bmatrix} \begin{bmatrix} Y_{1} \\ C & -X_{2} \end{bmatrix} \begin{bmatrix} R_{2} \\ R_{3} \end{bmatrix} m$

[0041]

(式中、

全ての置換基および変数はこれまでに記載したとおりである)。上記の多数の末端分枝が ビス系においても等しく適用されることが分かるであろう。

[0042]

本発明の式が含んでなるその他の置換基および変数に関しては、以下のものが好ましい: Y₁₋,は各々、酸素であり;

 R_{2-10} および R_{12} は各々、好ましくは水素または低級アルキル、例えば、 C_{1-6} であり;

Rı、RıおよびR₄は好ましくは-OH。であり;

(m)は1または2であり;

(n)および(p)は各々、0または1~4の整数のいずれかであり;

(v)は0または1であり;

(t)は1であり;

L, は-(CH, CH, O), -であり; かつ

 $\begin{array}{l} \mathsf{L}_1 \& -\mathsf{CH}_2 - \mathsf{L}_2 & \mathsf{CO}_3 - \mathsf{L}_3 - \mathsf{CH}_3 - \mathsf{L}_3 - \mathsf{CH}_3 - \mathsf{CO}_3 - \mathsf{CH}_3 -$

[0043]

B.<u>Ar成分の説明</u>

式(I)に関して、Ark式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であると考えられる。重要な特徴はArk成分が本質的に芳香性であることである。一般に、芳香族であるには、環分子面の上下にある「雲」内で π 電子が共有される必要がある。さらに、 π 電子数はヒュッケル則(4n+2)に従うものでなければならない。当業者ならば、無数の成分がその成分の芳香族必要条件を満たし、それゆえ本発明における使用に好適であることが分かるであろう。1つの特に好ましい芳香族基は:

【化17】

[0044]

(式中、

 R_{18-20} は R_{11} の定義と同じ基から選択される)である。その他の芳香族基としては:

【化18】

$$R_{1B}$$
 Z_2
 Z_1
 Z_2
 Z_1
 Z_2
 Z_1
 Z_2
 Z_1

R₁₈

Z₃ R₁₁

[0045]

试中、

 Z_1 および Z_2 は独立に、 $CR_{2,2}$ または $NR_{2,1}$ であり;かつ Z_1 はO、Sまたは $NR_{2,1}$

(式中、

 R_{18-22} は R_{11} の定義と同じ基またはシアノ、ニトロ、カルボキシル、アシル、置換アシルもしくはカルボキシアルキルから選択される)

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である}

が挙げられる。ベンゾおよびジベンゾ系のほか、5および6員環を有する異性体もまた包含され、それらの関連同族体もまた包含される。また当業者ならば、ヒュッケル則に従いさえすれば、所望により、芳香環がO、S、NR、1などのヘテロ原子で置き換えられてもよいことが分かるであろう。さらに、所望により、芳香族または複素環式構造が当技術分野で一般的に理解されているハロゲンおよび/または側鎖で置き換えられてもよい。また、本発明のAr成分に好適な全ての構造はB、またはB、含有基および(R、1)基を同一平面のオルト位に存在させることが可能である。

[0046]

C.プロドラッグの加水分解による薬物生成

本発明のプロドラッグ化合物は、血漿中の加水分解t,/,が排出t,/,よりも短くなるようにように設計される。

[0047]

治療を受けている哺乳類の血漿中における本発明の化合物に含まれる結合の加水分解 $t_{1/2}$ は、排出前に、十分な量の親化合物(すなわち、アミノまたはヒドロキシル基含有生物活性化合物)を放出させるのに十分短い加水分解 $t_{1/2}$ である。本発明のいくつかの好ましい化合物の血漿中における加水分解の $t_{1/2}$ は約5分~約12時間の範囲である。好ましくは、組成物の血漿加水分解 $t_{1/2}$ が約0.5~約8時間の範囲、さらに最も好ましくは約1~約6時間の範囲である。

[0048]

D. 実質的に非抗原性である高分子

上記のように、R₂はポリアルキレンオキシド(PAO)またはポリエチレングリコール(PEG)などの好ましくは実質的に非抗原性である水溶性高分子残基である。本発明の好ましい態様では、R₂は本明細書においてAとよばれる、二官能性またはビス−高分子系を形成しうる上記のキャッピング基をさらに含む。

[0049]

例として、本発明の組成物のPEG残基部分は、限定されるものではないが、次の:

- -C(=Y₆)-(CH,), -0-(CH, CH, 0), -A,
- -C(=Y,)-Y, -(CH,), -0-(CH, CH, 0), -A,
- $-C(=Y_6)-NR_2, -(CH_2)_6-0-(CH_2CH_2O)_x-A$
- -(CR, 4R, ,), -0-(CH,), -0-(CH, CH, 0), -A,
- -NR, , -(CH,), -0-(CH, CH, O), -A,
- $-C(=Y_6)-(CH_3)_4-0-(CH_3CH_3O)_4-(CH_3)_4-C(=Y_6)-$
- -C(=Y₆)-Y₇-(CH,)₆-O-(CH, CH, O)₇-(CH,)₇-Y₇-C(=Y₆)-\
- $-C(=Y_{6})-NR_{2}, -(CH_{5})_{6}-O-(CH_{5}CH_{5}O)_{4}-(CH_{5})_{6}-NR_{2}, -C(=Y_{6})-C(=Y_{6})_{7}$
- -(CR, 4 R, 、)。-O-(CH,), -O-(CH, CH, O)、-(CH,), -O-(CR, 4 R, 、)。-、および
- $-NR_{23}$ -(CH₂)_f -0-(CH₂CH₂O)_x -(CH₂)_f -NR₂₃ -

(式中、

Y₆およびY₇は独立に、O、SまたはNR₂3であり;

×は重合度であり;

 R_{23} 、 R_{24} および R_{25} は独立に、H、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{1-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} へテロアルキル、置換 C_{1-6} へテロアルコキシ、フェノキシおよび C_{1-6} へテロアルコキシからなる群から選択され;

eおよびfは独立に、0、1、または2であり; かつ

Aはキャッピング基である)

から選択されうる。

[0050]

高分子の重合度(x)は約10~約2,300でありうる。これは高分子鎖の繰り返し単位数を示す ものであり、高分子の分子量に依存している。(A)部分は本明細書において記載するキャ

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ッピング基、すなわち、高分子の末端に見られる基であり、いくつかの態様では、H、NH、 $OH、CO_2H、<math>G_{-6}$ アルキルまたは当業者によって理解されているその他のPEG末端活性化基のいずれかから選択されうる。

[0051]

また、ポリプロピレングリコール、本願出願人による米国特許第5,643,575号で記載されたものなどの分枝PEG誘導体、Shearwater Polymers, Inc. カタログ "Polyethylene Glyc ol Derivatives 1997–1998"で記載されたものなどの「星型PEG」および分岐したPEGも有用である。なお、上記の各々の開示は参照により本明細書に組み入れる。要すれば、過度の試験を行うことなく、二官能性結合基との結合のために水溶性高分子を官能化しうることが分かるであろう。

[0052]

さらなる実施形態では、R₁は所望により、1種以上のデキストラン、ポリビニルアルコール、炭水化物系高分子、ヒドロキシプロピルメタクリルアミド、ポリアルキレンオキシドおよび/またはそのコポリマーから選択されてもよい。本願出願人による米国特許第6,153,655号も参照されたい。なお、その内容は参照により本明細書に組み入れる。

[0053]

本発明の多くの態様では、複数置換高分子複合体が望まれる場合にはビス-活性化ポリエチレングリコールが好ましい。また、一置換高分子が望まれる場合にはポリエチレングリコール(PEG)、モノメチル基を末端にもつポリエチレングリコール(mPEG)などのモノ活性化G-4アルキル基を末端にもつポリアルキレンオキシド(PAO)が好ましい。

[0054]

所望の加水分解可能な結合を提供するためには、モノまたはジPEGアミンおよびモノまたはジPEGジオールのほか、PEG酸またはPEG二酸などの一または二酸活性化高分子も使用できる。好適なPAO酸はまずmPEG-OHをエチルエステルに変換し、その後、鹸化することにより合成できる。Gehrhardt、H.,ら、Polymer Bulletin 18: 487 (1987)およびVeronese、F. M.,ら、J. Controlled Release 10; 145 (1989)も参照されたい。また、PAO酸はmPEG-OHをt-ブチルエステルに変換し、その後、酸開裂することにより合成できる。例えば、本願出願人による米国特許第5,605,976号を参照されたい。なお、上記の各々の開示は参照により本明細書に組み入れる。

[0055]

PAOおよびPEGは平均分子量の点で実質的に異なりうるが、本発明のほとんどの態様においてプロドラッグの高分子部分の重量平均分子量は少なくとも約20,000である。好ましくは、R₁の重量平均分子量は約20,000~約100,000、さらにより好ましくは約25,000~約60,000である。プロドラッグに含有させるのに選択される高分子の平均分子量は、リンカーの加水分解前に、プロドラッグの十分な循環を提供するのに十分なものでなければならない

[0056]

本明細書において包含される高分子物質は、好ましくは室温で水溶性である。限定されるものではないが、かかる高分子としては、ポリエチレングリコール(PEG)またはポリプロピレングリコールなどのポリアルキレンオキシドホモポリマー、ポリオキシエチレン化ポ 40リオール、およびそのコポリマー、ならびにブロックコポリマーの水溶性が維持される場合にはそのブロックコポリマーが挙げられる。

[0057]

PEGなどのPAOについて本明細書において記載したように同様の活性化が行われるなら、デキストラン、ポリビニルアルコール、炭水化物系高分子、ヒドロキシプロピルメタクリルアミド(HPMA)、およびそのコポリマーなどのような有効に非抗原性な材料をPAO系高分子の代わりとして使用できる。当業者ならば、上記のリストは例示にすぎず、本明細書において記載する性質を有する全ての高分子材料が包含されることが分かるであろう。本発明の目的では、「有効に非抗原性」および「実質的に非抗原性」とは、当技術分野において実質的に毒性がなく、かつ哺乳類において感知できる免疫応答を誘導しないと認識される50

全ての高分子材料を包含するものと理解される。

[0058]

ポリプロピレングリコール酸など上記のもの以外のポリアルキレンオキシド誘導体、ならびにその他の二官能性結合基もまた包含されることは上記の説明から明らかであろう。

[0059]

E. プロドラッグ候補

1.ヒドロキシル基含有化合物の残基

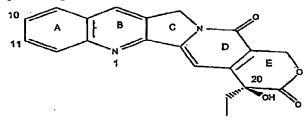
a.カンプトテシンおよび関連トポイソメラーゼI阻害剤

カンプトテシンは中国で自生するカンプトテカ・アクミナタ (Camptotheca accuminata)の樹木およびインドで自生するクサミズキ (Nothapodytes foetida)の樹木で産生される水に 10 不溶性の細胞傷害性アルカロイドである。カンプトテシンおよび関連化合物ならびに類似体は有望な抗癌または抗腫瘍剤であることも知られており、さらにこれらの活性がin vit roおよびin vivoにおいて発揮されることも分かっている。また、カンプトテシンおよび関連化合物は本発明のプロドラッグへの変換候補でもある。

[0060]

カンプトテシンおよび特定関連類似体は共通した構造:

【化19】



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[0061]

を有している。

 $[0\ 0\ 6\ 2\]$

この主要構造から、いくつかの公知な類似体が製造されてきた。例えば、A環はOHで10および11位のいずれかまたはその両方を置換しうる。また、A環は直鎖または分枝鎖 C_{1-30} アルキルまたは C_{1-17} アルコキシ(所望により、ヘテロ原子、すなわち、Oまたは C_{1-30} で現と結合していてもよい)で9位も置換しうる。B環は直鎖もしくは分枝鎖 C_{1-30} アルキルもしくは置換アルキル、 C_{1-30} アルコキシ、フェニルアルキルなど、アルキルカルバメート、アルキルカルバジド、フェニルヒドラジン誘導体、アミノ、アミノアルキル、アラルキルなどで7位を置換しうる。 C_{1-30} アルコキシ、フェニルアルキルなどで7位を置換しうる。 C_{1-30} アルコキシ、フェニルアルキルなどで7位を置換しうる。 C_{1-30} アルコキシ、フェニルアルキルなど、アルキルカルバジド、フェニルヒドラジン誘導体、アミノ、アミノアルキル、アラルキルなどで7位を置換しうる。 C_{1-30} アルコキシ、フェニルアルキルなど、アルキルカルバジド、フェニルヒドラジン誘導体、アミノ、アミノアルキル、アラルキルなどで7位を置換しうる。 C_{1-30} アルコキシ、フェニルアルキルなど、アルキルカルバジド、フェニルアルキルなど、アルキルカルバジド、フェニルアルキルなど、アルキルカルバジド、フェニルアルキルなどで7位を置換したる。本発明における使用に好ましいカンプトテシン誘導体としては、20-OHまたは本明細書において記載する活性化型高分子輸送系と直接反応しうるまたは後にPEGなどの高分子と結合する結合部分中間体、例えば、イミノ二酢酸などと反応しうるもう1つのOH基を含むものが挙げられる。本明細書において記載したカンプトテシン類似体は例示を目的とするものであって、これに限定されない。

[0063]

b. <u>タキサン系化合物およびパクリタキセル</u>誘導体

本発明のプロドラッグ組成物に含められる化合物種の1つがタキサン系化合物である。本発明の目的では、「タキサン」とは、タキサン系テルペンに入る全ての化合物を包含するものである。よって、タキソール(パクリタキセル)、3'-置換 tert-ブトキシ-カルボニル-アミン誘導体(タキソテール)など、ならびに標準有機技術を用いて容易に合成されるまたはSt. Louis, MissouriのSigma Chemicalなどの民間供給会社から入手可能であるその他の類似体は本発明の範囲である。これらの誘導体は有効な抗癌剤であることが分かって 50

いる。多くの研究により、これらの薬剤が数種類の悪性腫瘍に対する活性を有することが示されている。現在まで、それらの使用には、特に、それらの供給が不足しており、水溶性が乏しく、さらに過敏症を引き起こす傾向があることから厳しい制限があった。本願出願人による米国特許第5,622,986号および第5,547,981号で開示された7-アリール-カルバメートおよび7-カルバザートをはじめとするその他のタキサン系化合物もまた本発明のプロドラッグに含めうることは理解すべきである。なお、上記米国特許の内容は参照により本明細書に組み入れる。パクリタキセルは好ましいタキサンである。

[0064]

C. さらなる生物活性成分

上記分子のほか多くの化合物を用いて本発明のプロドラッグ製剤が製造できる。例えば、 10ビス-PEG複合体などの生物学上活性な化合物が、

ゲムシタビン:

【化20】

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[0065]

または

ポドフィロトキシン:

【化21】

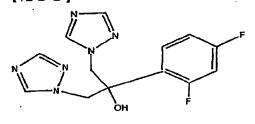
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[0066]

または

フルコナゾールなどのトリアゾール系抗真菌薬:

【化22】



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[0067]

または

シクロピロックス:

[0068]

または

Ara-C:

【化24】

[0069]

などの化合物から誘導された。

[0070]

本発明の高分子系プロドラッグは特にかかる水不溶性化合物を送達するのに十分に好適な ものであるが、プロドラッグ形態用に選択される親化合物が実質的に水不溶性である必要 はない。その他の有用な親化合物としては、例えば、生物学上活性な特定の低分子量タン パク質、酵素およびペプチドグリカンをはじめとするペプチド、ならびにその他の抗腫瘍 剤;フォルスコリンなどの心血管作動薬;コンブレタスタチン、ビンブラスチン、ドキソ 30 ルビシン、メイタンシンなどの抗新生物薬; バンコマイシン、エリスロマイシンなどの抗 感染症薬;ナイスタチン、アムホテリシンB、トリアゾール、パピュロキャンディン、ニ ユーモキャンディン、エキノキャンディン、ポリオキシン、ニッコーマイシン、プラジミ シン、ベナノミシンなどの抗真菌薬 ("Antibiotics That Inhibit Fungal Cell Wall Dev elopment"Annu. Rev. Microbiol. 1994, 48: 471-97(その内容は参照により本明細書に 組み入れる)を参照されたい);抗不安薬、胃腸薬、中枢神経系活性化剤、鎮痛剤や排卵 誘発剤または避妊薬、抗炎症薬、ステロイド系薬剤、抗尿酸血症薬、心血管作動剤と血管 拡張薬、血管収縮薬などが挙げられる。

[0071]

上記のものは本発明のプロドラッグに好適である生物学上活性な成分の例示である。特記 40 していないが、好適なエステル形成基、すなわち、ヒドロキシル基を有する生物学上活性 な材料もまた本発明の範囲とされるものと理解すべきである。また、本発明のプロドラッ グ複合体が、1当量の薬物および高分子だけでなくin vivoにおいて生物活性に影響を及ぼ さない成分を含有する少量の化合物も含有してよいことも理解すべきである。例えば、い くつかの例では、二酸と1個の結合ポイントを有する薬物分子とを反応させても、その反 応条件では高分子当たり2当量の薬物を有するプロドラッグが定量的な量で生成されない ということが分かっている。カルボジイミドを使用する場合にはアシル尿素などの反応副 生成物が生じる場合がある。

[0072]

2.<u>アミン基含有化合物の残基</u>

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本発明のいくつかの態様では、B₁またはB₂はアミン基含有化合物の残基である。限定されるものではないが、かかる好適な化合物としては、有機化合物、酵素、タンパク質、ポリペプチドなどの残基が挙げられる。有機化合物としては、限定されるものではないが、ダウノルビシン、ドキソルビシン; p-アミノアニリンマスタード、メルファラン、Ara-C(シトシンアラビノシド)などをはじめとするアントラサイクリン系化合物、および関連代謝拮抗性化合物、例えば、ゲムシタビン、などの成分が挙げられる。あるいは、Bはアミン基含有心血管作動剤、抗新生物薬、抗感染症薬、ナイスタチンおよびアムホテリシンBなどの抗真菌薬、抗不安薬、胃腸薬、中枢神経系活性化剤、鎮痛薬、排卵誘発剤、避妊薬、抗炎症薬、ステロイド系薬剤、抗尿酸血症薬、血管拡張薬、血管収縮薬などの残基でありうる。

[0073]

本発明の好ましい態様では、アミノ基含有化合物は、動物、例えば、ヒトをはじめとする 哺乳類のかかる治療が望まれる症状の治療における医薬上のまたは診断上の使用に好適な 、生物学上活性な化合物である。上記のものは例示を意図するものであり、改変しうる化合物を限定するものではない。当業者ならば、その他のかかる化合物も過度な試験を行うことなく同様に改変しうることが分かるであろう。特に示していないが、好適なアミノ基を有する生物学上活性な材料もまた本発明の範囲とされるものと理解すべきである。

[0074]

本発明において含有させるのに好適なアミノ基含有分子のタイプについての唯一の条件は、担体部分と反応しかつそれと結合しうる利用可能な少なくとも1つの(第1または第2)アミン基含有位置が存在することと、プロドラッグ系が親化合物を放出して、親化合物を再生利用した後に生物活性の実質的な喪失がないことである。

[0075]

本発明のプロドラッグ組成物への含有に好適な親化合物は、それ自体が結合型組成物からの加水分解による放出後は活性ではないが、さらなる化学工程/反応を受けた後に活性となりうる物質/化合物であってよいことに注目されたい。例えば、ダブルプロドラッグ輸送系によって血流に送達される抗癌剤は、癌または腫瘍細胞に浸透するまで不活性な状態にあり、そこで癌または腫瘍細胞化学、例えば、その細胞に特異的な酵素反応により活性化されると考えられる。

[0076]

3.脱離基

B,またはB,が脱離基である態様では、好適な脱離基としては、限定されるものではないが、N-ヒドロキシベンゾトリアゾリル、ハロゲン、N-ヒドロキシフタルイミジル、p-ニトロフェノキシ、イミダゾリル、N-ヒドロキシスクシンイミジル;チアゾリジニルチオンなどの基を挙げることができ、あるいは当業者には理解されるその他の好適な脱離基が挙げられる。本明細書において使用し記載する合成反応は過度の試験を行わなくとも当業者ならば分かるであろう。

[0077]

例えば、化合物(I)のアシル化中間体をクロロ蟻酸4-ニトロフェニル、ジスクシンイミジルカーボネート(DSC)、カルボニルジイミダゾール、チアゾリジンチオンなどの反応物質と反応させて所望の活性化誘導体を得ることができる。

[0078]

p-ヒドロキシベンジルアルコールまたはp-アミノベンジルアルコール、およびo-ヒドロキシベンジルアルコールまたはo-アミノベンジルアルコールのフェノールまたはアニリン部分の選択的アシル化は、例えば、チアゾリジンチオン活性化高分子、スクシンイミジルカーボネート活性化高分子、カルボン酸活性化高分子、プロックアミノ酸誘導体を用いて行うことができる。適切に実施されれば「活性化」型PEGプロドラッグ(またはブロックプロドラッグ)はアミンまたはヒドロキシル基含有化合物との複合体化が可能である。

[0079]

F.<u>高分子プロ</u>ドラッグ輸送系<u>の合成</u>

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典型的な高分子プロドラッグの合成を実施例で記載するが、一般に、プロドラッグ輸送系を製造する1つの好ましい方法では、最初に高分子残基を分枝基に結合させる。別途、生物学上活性な成分または薬物、例えば、薬物-OHまたは薬物-NH、(式IのB1またはB2)をTML成分に結合させる。このTML成分は、高分子との結合ポイントに二官能性スペーサーを含んでもよい。次に、末端分枝を有する高分子残基と薬物-TML成分とを最終生成物を生成するのに十分な条件下で反応させる。

[0080]

二官能性スペーサーを含有するTML-薬物成分と高分子部分との結合は、好ましくはカップリング剤の存在下で行われる。限定されるものではないが、好適なカップリング剤としては、例えば、Sigma-Aldrich Chemicalなどの民間供給会社から入手可能である、または公 10 知の方法により合成される1,3-ジイソプロピルカルボジイミド(DIPC)、好適なジアルキルカルボジイミド、ハロゲン化2-ハロ-1-アルキル-ピリジニウム、(Mukaiyama試薬)、1-(3-ジメチルアミノプロピル)-3-エチルカルボジイミド(EDC)、プロバンホスホン酸環状無水物(PPACA)およびジクロロリン酸フェニルなどが挙げられる。

[0081]

好ましくは、置換基を塩化メチレン、クロロホルム、DMFまたはその混合物などの不活性 溶媒中で反応させる。この反応は、好ましくは生成した全ての酸を中和するためにジメチ ルアミノピリジン、ジイソプロピルエチルアミン、ピリジン、トリエチルアミンなどの塩 基の存在下、O℃~約22℃(室温)の温度で行われる。

[0082]

より詳細には、高分子輸送系を製造する1つの方法は式(VIII):

【化25】

$$H-J$$
 L_{1}
 L_{2}
 L_{2}
 L_{2}
 L_{2}
 L_{3}
 L_{4}
 L_{1}
 L_{2}
 L_{2}
 L_{3}
 L_{4}
 L_{4}
 L_{1}
 L_{2}
 L_{4}
 L_{4}
 L_{4}
 L_{4}
 L_{5}
 L_{5}

[0083]

全ての置換基および変数はこれまでに定義したとおりであり、かつ B', はヒドロキシルまたはアミン基含有成分の残基である) で表される化合物と式(IX):

【化26】

(IX)

[0084]

{式中、

R,は高分子残基であり;

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Y, はO、SまたはNR、であり、

MはO、SまたはNR,であり;

(a)は0または1であり;

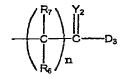
(m)は0または正の整数であり;

Y₂₋, は独立に、O、SまたはNR₁, であり;かつ

 R_{2-3} は独立に、水素、 Q_{-6} アルキル、 Q_{3-12} 分枝鎖アルキル、 Q_{-8} シクロアルキル、 Q_{-6} 置換アルキル、 Q_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 Q_{-6} ペテロアルキル、置換 Q_{-6} ペテロアルキル、 Q_{-6} ペテロアルキシ、フェノキシおよび Q_{-6} ペテロアルコキシからなる群から選択され:

E, は

【化27】

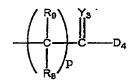


[0085]

であり;

 E_{6-8} は独立に、H、 E_{5} または

【化28】



[0086]

(中、

D,およびD,は独立に、OHまたは保護されていないアミンまたはヒドロキシルと反応しうる脱離基、または末端分枝基であり;

(n)および(p)は独立に、0または正の整数であり;

Y2-3は独立に、O、SまたはNR10であり;かつ

 R_{6-10} は独立に、水素、 C_{1-6} アルキル、 C_{5-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{1-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} へテロアルキル、置換 C_{1-6} へテロアルキル、 C_{1-6} アルコキシからなる群から選択される)

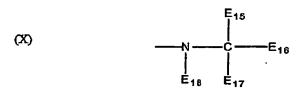
である}

で表される化合物とを反応させることを含む。

[0087]

この方法のさらなる態様では、D,およびD,は独立に、式〇〇

【化29】



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[0088]

(式中、

 $E_{1,-1}$ 。は D_3 および D_4 が以下で定義する D_3 、および D_4 、に変わることを除き、 $E_{1,-1}$ の定義と同じ基から選択される)

で表される選択された末端分枝基である。この実施形態では、D',およびD',が独立に、OH、式(IV)または(V)で表される成分、または(XI)

[化30]



. [0089]

(式中、

 E_{5-28} は D_3 および D_4 が D_3 がよび D_4 に変わり、かつ D_3 および D_4 が独立に、 D_4 に変わり、かつ D_3 および D_4 が独立に、 D_4 に変わり、かつ D_3 が独立に、 D_4 が独立に、 D_5 00円であるにはははいる。 D_5 1の定義と同じ基から選択される)

で表される成分でありうる。

[0090]

かかる合成方法により、最大16当量のカルボン酸または活性化カルボン酸を、例えば、結合させることが可能である。本明細書における好ましい構造で示されるように、末端分枝多酸を有するPEG残基が本発明の好ましい態様である。

[0091]

選択された合成方法にかかわらず、本明細書において記載する合成方法によって得られる 好ましい化合物としては、

【化31】

【0092】 および 【化32】

[0093]

(式中、

R₁はPAOまたはPEGなどの高分子残基であり、かつDはOH、式(IV)または(V)である。好まし 20 くは、Dは 【化33】

【0094】 または 【化34】

[0095]

(式中、

Bはアミンまたはヒドロキシル基含有薬物の残基である)

である}

が挙げられる。

[0096]

本発明のもう1つの好ましい態様では、本発明の化合物は式(XII):

【化35】

[0097]

、中、

全ての置換基および変数はこれまでに定義したとおりである)

で表される。

[0098]

G.in vivo診断学

本発明のさらなる態様は、所望により、診断または造影目的に選択される診断タグを上記 の輸送エンハンサーに付けて作製してもよい本発明の複合体を提供する。そのため、好適 なタグは、好適な成分、例えば、アミノ酸残基を、当技術分野の標準放射性同位元素、放 射線不透過性標識、磁気共鳴標識、またはその他、磁気共鳴映像法に好適なその他の非放 射性同位元素標識、蛍光標識、外科処置中の腫瘍組織のイメージングを可能にする可視色 を呈する標識および/または紫外線、赤外線または電気化学的刺激下で蛍光発光可能な標 識などに結合させることにより作製される。所望により、診断タグを複合体化される治療 成分に組み込みおよび/または結合させて、動物またはヒト患者内での治療用生物活性材 料の分布のモニタリングを可能にすることができる。

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[0099]

本発明のなおさらなる態様では、本発明のタグ付き複合体が当技術分野で公知な方法により、例えば、放射性同位元素標識をはじめとする好適な標識を用いて容易に作製される。一例として、これらにはin vivoにおいて腫瘍細胞に選択的に取り込まれる放射免疫シンチグラフ用薬剤を製造するための¹³¹ヨウ素、¹²⁵ヨウ素、⁹⁹ⁿテクネチウムおよび/または¹¹¹インジウムが挙げられる。例えば、一例として、参照により本明細書に組み入れる米国特許第5,328,679号;同第5,888,474号;同第5,997,844号;および同第5,997,845号により示されたものをはじめとする、ペプチドをTc-99mに結合させる当技術分野で公知な方法が多数ある。

[0100]

一般には、患者の腫瘍組織の解剖学的位置決定では、腫瘍を有することが予測される患者または動物に複合体タグを投与する。標識化免疫グロブリンを腫瘍部位に位置付けるのに十分な時間が経過した後、標識により発生するシグナルを、例えば、X線ラジオグラフィー、コンピュータ体軸横断X線断層撮影、MRIにより、発光性タグの機器検出により、ガンマカメラなどのフォトスキャン装置、または選択されたタグの性質に好適なその他の方法もしくは装置により視覚的に検出する。

[0101]

次いで、検出されたシグナルを画像または腫瘍部位の解剖学的および/もしくは生理学的 判定に変換する。この画像によりin vivoにおける腫瘍の位置付けが可能になり、好適な 治療計画の立案ができる。タグ付き成分自体が治療薬である実施形態では、検出されたシ ²⁰ グナルによって治療中の解剖学的位置決定が明らかであり、診断的および治療的インター ベンションを追跡するための基準が提供される。

[0102]

H.治療方法

本発明のもう1つの態様により、哺乳類における種々の病状に向けた治療方法が提供される。これらの方法は、かかる病状の治療が必要な哺乳類に、有効量の、本明細書において記載するように製造したマルチ・ローディッド(multi-loaded)Ara-C-PEG複合体などのプロドラッグを投与することを含む。該組成物は特に、新生物性疾患を治療する、全身腫瘍組織量を減少させる、新生物転移を予防する、および哺乳類における腫瘍/新生物増殖の再発を予防するのに有用である。

$[0\ 1\ 0\ 3\]$

投与するプロドラッグ量はその中に含まれる親分子に応じたものとなる。一般に、治療方法に使用するプロドラッグ量は哺乳類において所望の治療効果を効果的に達成する量である。必然的に、種々のプロドラッグ化合物の投与量は親化合物、in vivo加水分解速度、高分子の分子量などによっても多少変わるが、一般には、タキサン系プロドラッグはタキサン部分の量を基に1日当たり約5~約500mg/m²の範囲の量で投与される。また、カンプトテシンプロドラッグも1日当たり約5~約500mg/m²の範囲の量で投与される。上記の範囲設定は例示であり、臨床経験および治療適用に基づき、選択されたプロドラッグの最適投与量が当業者により決定されるであろう。実際の投与量については必要以上の試験を行うことなく、当業者には明らかであろう。

[0104]

哺乳類へ投与するために本発明のプロドラッグを1種以上の好適な医薬組成物に含めることができる。医薬組成物は当技術分野で十分に公知な方法に従って製造される液剤、懸濁剤、錠剤、カプセル剤などの形態であってよい。また、当業者が要すれば、かかる組成物の投与は経口および/または非経口経路によるものであってよいと考えられる。組成物の溶液および/または懸濁液は、例えば、当技術分野で公知な方法、例えば、静脈内、筋肉内、皮下注射などによる組成物の注入または浸潤用の担体ビヒクルとして使用しうる。【0105】

また、かかる投与は体内スペースもしくは体腔への注入、ならびに吸入および/または経 鼻経路によるものであってもよい。しかしながら、本発明の好ましい態様では、プロドラ

ッグはその必要のある哺乳類に非経口投与される。

【実施例】

[0106]

I.実施例

次の実施例は本発明をさらに理解するためのものであり、本発明の有効な範囲を何ら制限するものではない。実施例で列挙される下線を施した太字体の数字は図1~5で示されるものと対応している。

[0107]

概説

反応は全て乾燥窒素またはアルゴン雰囲気下で行った。市販の試薬はさらなる精製を行わ 10 ずに使用した。全てのPEG化合物は使用前に真空下または共沸蒸留(トルエン)により乾燥させた。 1 Hスペクトルは特に断りのない限り、溶媒としてジュウテリオクロロホルムを用いてJEOL FT NMR システム JNM GSX-270装置で測定した。 1 C NMRスペクトルはJNM GSX-2 70では67.80MHzで測定した。化学シフト(σ)はテトラメチルシラン(TMS)から低磁場へ向かう百万分の一(ppm)単位で表され、結合定数(J値)はヘルツ(Hz)で示される。in vivo薬物処理前の注入用に全てのPEG複合体化化合物を減菌生理食塩水(0.9%)に溶解し(\sim 15mg/m L)、それらをara-C等価物として投与した(絶対量のara-Cを投与)。

[0108]

HPLC法

C8逆相カラム(Beckman, ultrasphere)を用い、移動相としてメタノール-水の80:20混合物 20 (v/v)を用いる定組成条件下でHPLC分析を行った。ピーク溶出は紫外吸光検出器を用いて2 54mmでモニターした。遊離PEGの存在を検出し、さらにPEG化生成物の存在も確認するため、蒸発光散乱検出器(ELSD)、PL-EMD 950型(Polymer Laboratories)を使用した。ELSDおよびUV分析では、全ての最終PEG化生成物には非改変薬物が含まれず、HPLCによる純度は≥9 5%であった。

[0109]

PEC誘導体中のAra-C含量の分析

PEG誘導体中のara-C含量を測定するため、 N^{ϵ} -アセチルシチジンを標準として使用した。 H_{2} 0中の N^{ϵ} -アセチルシチジンのUV吸光度を 0.01μ mol/mL \sim 0.05 μ mol/mLの範囲にわたる6種の異なる濃度で257nmにおいて測定した。濃度に対する吸光度の検量線での N^{ϵ} -アセチルシチジンの吸光係数 ϵ の計算値は36.4であった(1mg/mLの257nmにおける0.D.、光路長1.0cm)。 PEG化ara-C誘導体をおよその濃度 0.015μ mol/mL(分子量40kDaを基にして)で10に溶解し、これら化合物のUV吸光度を257nmで測定した。この値と、さらに上記で得た吸光係数 ϵ を用いて、サンプル中のara-C濃度を調べた。この値をサンプル濃度で割ってサンプル中のara-Cの割合(%)を求めた。

[0110]

PEG誘導体中のメルファラン含量の分析

PEG誘導体中のメルファラン含量を測定するため、メルファランを標準として使用した。D MF-H, O(9:1, V/V)中のメルファランのUV吸光度を $0.02\,\mu$ mol/mL $\sim 0.06\,\mu$ mol/mLの範囲にわたる5種の異なる濃度で264nmにおいて測定した。濃度に対する吸光度の検量線でのメルファランの吸光係数 ϵ の計算値は54.6であった (1mg/mLの264nmにおける0.0.、光路長1.0cm)。 PEG化メルファラン誘導体をおよその濃度 $0.013\,\mu$ mol/mL(分子量40kDaを基にして)でDMF-H, 0 (9: 1, V/V)に溶解し、これら化合物のUV吸光度を264nmで測定した。この値と、さらに上記で得た吸光係数 ϵ を用いて、サンプル中のメルファラン濃度を調べた。この値をサンプル濃度で割ってサンプル中のメルファランの割合(%)を求めた。

[0111]

略語

DCM(ジクロロメタン)、DMAP(4-(ジメチルアミノ)ピリジン)、<math>EDC(1-x+y-3-(3-3)+y-3-(3-3)+y-3-(3

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[0112]

[実施例1] 化合物3a

無水ピリジン(50mL)中のara—C(1, 1.73g, 7.12mmol)、2a(700mg, 1.78mmol)、HOBT(0.96g , 7.12mmol)、およびEDC・HCl(2.73g, 14.25mmol)の混合物を室温で2時間攪拌し、温度を40℃に上昇させてこの反応を一晩続けた。溶媒を除去し、塩化メチレン(50mL)を用いて混合物を溶解した後、水(3×30mL)、次ぎに、0.1N HCl(2×30mL)で洗浄した。有機層を無水MgSO4で乾燥させ、溶媒を真空除去すると粗生成物が得られた。これをシリカゲルカラムクロマトグラフィー(DOM中5~10%MeOH)により精製し、638.8mg(52%)の3aを白色の固体として得た: HNMR δ 1.42, 1.55, 2.17, 2.26, 2.46, 2.79, 3.84, 3.91, 4.14, 4.33, 4.53, 5.49, 6.07, 6.17, 6.52, 6.76, 7.31, 7.67, 8.16, 8.62; CNMR δ 17.77, 20.11 10, 25.36, 28.32, 31.51, 31.96, 39.57, 50.18, 50.45, 61.88, 74.50, 80.15, 85.90, 88.58, 96.25, 122.51, 132.82, 133.34, 136.73, 138.22, 146.57, 149.90, 155.65, 155.96, 162.08, 171.89, 174.06。

[0113]

[実施例2] 化合物3b

化合物1を実施例1と同様の条件を用いて2bと結合させ、3bを54%の収率で得た: 13 C NMR $_{0}$ _17.23, 17.92, 18.33, 25.49, 28.32, 31.51, 31.58, 31.99, 32.46, 39.52, 40.09, 50.08, 50.22, 61.72, 74.50, 74.94, 80.11, 80.15, 85.45, 85.90, 88.01, 88.58, 96.25, 122.51, 128.77, 129.03, 129.16, 131.68, 132.82, 136.24, 136.73, 138.22, 146.05, 146.57, 149.90, 155.65, 155.96, 171.85, 171.89, 174.06。

[0114]

[実施例3] <u>化合物4a</u>

無水DOM(6mL)およびTFA(4mL)中、化合物3a(638.8mg, 1.03mmo1)を室温で2時間攪拌した。この溶液にエチルエーテルを加えると粗生成物が沈殿した。これを濾過し、エーテルで洗浄し、4aを白色の固体として得た(534.5mg, 82%): H NMR (DMSO-d₆) δ 1.52 (s, 3H, (C H₃), CH) 1.55 (s, 3H, (CH₃), CH), 1.62 (d, 1 H, J= 8.1 Hz, (CH₃), CH), 2.22 (s, 3H, CH₃Ar), 2.57 (s, 3H, CH₃Ar), 2.97 (s, 2H, CH₄C(=0)), 3.41—4.27 (m, 5 H, ara—C's H—2'—H5'), 6.09 (d, 1H, J = 5.4, ara—C's H—1'), 6.67 (s, 1H, Ar—H), 6.90 (s, 1H, Ar—H), 7.12 (d, J= 5.4, H—6), 8.05 (d, J= 8.1, H—5), 8.67 (bs, 1H, TFA); C NM R (DMSO-d₆) δ 15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61 30.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.68, 171.87。

[0115]

[実施例4] 化合物4b

化合物3bを実施例3と同じ条件に付し、4bを82%の収率で得た: H NMR (DMSO-d₆) δ _1.52 (s, 3H, (口⅓), CH) 1.55 (s, 3H, (口⅓), CH), 1.62 (d, 1 H, J= 8.1 Hz, (CH₃), CH), 2. 22 (s, 3H, CӇ₃Ar), 2.57 (s, 3H, CӇ₃Ar), 2.97 (s, 2H, CӇ₂C(=O)), 3.41—4.27 (m, 5 H, ara—C's H—2'—H5'), 6.09 (d, 1H, J = 5.4, ara—C's H—1'), 6.67 (s, 1H, Ar—H), 6 .90 (s, 1H, Ar—H), 7.12 (d, J= 5.4, H—6), 8.05 (d, J = 8.1, H—5), 8.67 (bs, 1H, TFA); C NMR (DMSO-d₆) δ _15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53 40 , 49.02, 61.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08 , 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.68, 171.87

[0116]

[実施例5] 化合物6a

無水DOM(50mL)中のPEG-アスパラギン酸(分子量40,000, 5, 3 g, 0.074mmol)、4a(385.6mg , 0.74mmol)、NMM(240mg, 2.38mmol)、HOBT(120.5mg, 0.89mmol)、およびEDC・HC1(228.4 mg, 1.19mmol)の混合物を0℃で30分間攪拌した。この反応物を室温に温め、反応を3日間続け、濾過した。濾液を真空濃縮し、残渣をIPAで再結晶化させ、2.7g(90%)の生成物を得た。UVアッセイで測定した生成物中のara-C量は2.11重量%であった: 13 C NMR & 14.40, 1 50

9.22, 24.86, 31.17, 38.26, 38.90, 47.94, 48.67, 49.66, 60.17, 61.12, 61.90, 67.8 6-70.87 (PEG), 71.70, 74.50, 85.01, 87.53, 95.28, 121.39, 131.18, 132.68, 133.19, 134.77, 137.70, 145.26, 138.93, 155.23, 160.12, 161.56, 168.39, 170.72, 170.92, 171.27, 171.34

[0117]

「実施例6D 化合物6b

化合物4bを実施例5と同じ条件に付し、6bを88%の収率で得た。UVアッセイで測定した生成物中のara-C量は1.68重量%であった: 13 C NMR 3 15.12, 16.22, 24.52, 24.73, 29.55, 30.55, 31.15, 38.04, 38.59, 47.66, 49.16, 49.93, 50.18, 60.93, 61.12, 62.90, 69.44-71.59 (PEG), 71.70, 74.50, 84.78, 84.90, 87.53, 94.85, 127.60, 130.20, 135.51, 136.10, 141.70, 145.15, 147.50, 155.00, 161.20, 169.47, 170.62, 170.92, 171.27。【0 1 1 8】

[実施例7] 化合物9

PEGジオール(7,55g, 1.38mmol)を2時間トルエンと共沸させた後、回転蒸発により200mL の溶媒を除去した。この溶液を \sim 30℃に冷却し、トリホスゲン(0.544g, 1.83mmol)を固体として、次ぎに、無水ピリジン(0.434g, 5.49mmol)を加え、反応混合物を50℃で1時間攪拌した。Nーヒドロキシフタルイミド(8, 1.12g, 6.88mmol)および無水ピリジン(0.54g, 6.88mmol)をクロロ蟻酸混合物に加え、反応物を50℃でさらに2時間、次ぎに、室温で12時間攪拌した。この反応混合物を濾紙で濾過し、溶媒を真空除去し、生成物を塩化メチレン-エチルエーテル(1100mL, 8:2, \vee 0)で再結晶化させ、生成物を得た(50.9g, 92%): 13 C NMR 20 20 123.62, 128.10, 134.55, 152.00, 160.00。

[0119]

[実施例8] <u>PEG-cmc-Asp-O-t-Bu(11)</u>

化合物9(分子量40,000, 20g, 0.459mmol)およびアスパラギン酸ジt-ブチルエステルHCl(1 0, 1.0g, 3.55mmol)を無水DCMに溶解した後、DMAP(0.433g, 3.55mmol)を加えた。この溶液を一晩還流した後、エチルエーテル(1L)を加えて沈殿させた。濾過により固体を単離し、IPA(1L)で2回再結晶化させた。濾過ケーキをIPA(200mL)およびエーテル(200mL)で洗浄し、45℃で真空乾燥させた後に15.6g(78%)の生成物を得た:¹³ C NMR & 27.837 (CH₂ CO₂ C(CH₃)₃), 27.991 (CHCO₂ C(CH₃)₃), 37.752 (CHCH₂ CO₂), 50.800 (NHCH), 64.212 (OCH₂ CH₃ OC(=O)NH), 81.333 (CH₂ CO₂ C(CH₃)₃), 82.007 (CHCO₂ C(CH₃)₃), 155.924 (OCH₃ CH₄ OC(=O)N 30 H), 169.674 (CH₂ CO₂ C(CH₃)₃), 169.969 (CHCO₂ C(CH₃)₃)。

[0120]

[実施例9] PEG-cmc-Asp-OH(12)

化合物11(15g, 0.375mmol)をDCM(150mL)に溶解した後、TFA(75mL)を加えた。この溶液を室温で2時間攪拌し、ヘキサン(500mL)を加えて固体を沈殿させた。この固体をヘキサンでトリチュレートしてTFAを除去した後、冷却したDCM-エーテルで再結晶化させた。再結晶化させた固体をDCM(150mL)に再溶解し、水(150mL)で洗浄した。有機層を分離し、無水MgS O_4 で乾燥させ、真空濃縮し、エーテルで沈殿させ、12.4g(83%)の生成物を得た: $^{1.3}$ C NMR $_8$ 36.441 (CHCH, CO_2), 50.177 (NHCH), 64.390 (OCH, CH, OC(=0)NH), 81.333 (CH, CO_2 C(CH, O_3), 82.007 (CHCO $_2$ C(CH, O_3), 156.172 (OCH, O_4 OC(=0)NH), 171.944 (CH, O_4 CO, O_4 C(CH, O_3), 172.211 (CHCO $_4$ C(CH, O_3), O_4

[0121]

[実施例10] Boc-Asp-Asp-OMe(15)

EDC・HC1(2.47g, 12.86mmo1)を無水DOM(30mL)およびDMF(2mL)中のBocNH-アスパラギン酸(13, 1g, 4.29mmo1)、アスパラギン酸ジメチルエステル・HC1(14, 1.86g, 9.43mmo1)、およびDMAP(2.47g, 12.86mmo1)の混合物にO℃で加えた。この混合物を室温に一晩温めた。混合物を1N HC1で3回洗浄し、有機層を無水MgSO₄で乾燥させた後、溶媒を真空除去し、生成物を得た(2.0g, 90%):¹H NMR& 1.45 (s, 9H), 2.62-3.02 (m, 6H, 3 x CH), 3.70 (s, 6H, 2 x OCH₃), 3.74 (s, 3H, OCH₃), 3.75 (s, 3H, OCH₃), 4.50 (bs, 1H, CH), 4.85 (m, 2H, 2 x CH), 6.05 (d, J = 6.95 Hz, 1H, NH), 6.98 (d, J = 8.05 Hz, 1H, NH), 7 50

.57 (d, J = 7.69 Hz, 1H, NH)

[0122]

[実施例11] Asp-Asp-OMe(16)

化合物15(2.0g, 3.85mmo1)をDCM(30mL)およびTFA(15mL)に溶解し、溶液を室温で2時間攪拌した。溶媒を真空除去し、残渣をDCM-エーテルで2回再結晶化させ、生成物(1.74g, 87%)を白色の固体として得た: $^{1.3}$ C NMR $_{8}$ 35.52, 48.76, 50.12, 51.90, 51.96, 52.65, 114.59, 118.49, 168.43, 170.02, 170.92, 171.17, 171.40, 171.48。

[0123]

[実施例12] PEG-cmc-Asp-Asp-OMe(17)

DMAP(4.5g, 36.86mmol)を700mLの無水クロロホルム中の9(分子量40,000,74g,1.84mmol) 10 および16(9.83g,18.43mmol)の溶液に加えた。この反応混合物を窒素下、24時間還流した。反応物を室温まで冷却し、濃縮して1/4の量にした。粗生成物を2.5Lのエーテルで沈殿させ、濾過し、5.5LのIPA(65℃)で再結晶化させた。生成物を濾過し、新鮮なIPAで2回、新鮮なエーテルで2回洗浄し、40℃で一晩乾燥させ、59.0g(84%)の17を得た: 13 C NMR δ 35.344,36.931,48.082,48.208,50.835,51.509,52.239,61.045,63.953,68.854—72.056,155.538,170.102,170.369,170.453,170.734。

[0124]

「実施例13] PEG-cmc-Asp-Asp-OH(18)

化合物17 (51g, 1.26mmol)およびLiOH・H₂O(0.8g, 18.9mmol)を300mLの水に溶解し、溶液を室温で一晩攪拌した。1N HClを加えて溶液のpHを2.5に調整した。溶液をDCM(3×600mL) 20 で抽出し、有機層を合し、無水MgSO₄で乾燥させ、真空濃縮した。残渣をDCMエーテルで再結晶化させると生成物が得られた。これを濾過により回収し、40℃で一晩乾燥させ、38g(54%)のオクタ酸(octa-acid)を得た: 13 C NMR (D₂O) $_{\delta}$ 38.384, 39.704, 51.951, 54.465, 62.934, 67.105, 71.445-74.381 (PEG), 159.772, 173.831, 174.940, 176.359, 176.69 6。

[0125]

[実施例14] Mel-OMe(20)

メルファラン(19, 1.00g, 3.28mmol)を2,2-ジメトキシプロパン(65.59mL, 533.49mmol)に 懸濁した。この懸濁液にHCl水溶液(36%, 3.28mL)および無水メタノール(4mL)を加えた。 混合物を、溶液がわずかに褐変し始めるまで激しく攪拌しながら穏やかに加温還流した後 30 、室温で18時間攪拌した。反応混合物を真空濃縮し、残渣から粗生成物をエーデルで沈殿させた。固体を濾過し、エーテルで洗浄し、シリカゲルカラムクロマトグラフィー(CHCl, :MeOH = 9:1, $_{\rm V}$)により精製し、所望の生成物を得た(0.47g, 45%): 13 C NMR $_{\rm S}$ 39.751, 40.340, 51.912, 53.435, 55.803, 112.124, 126.076, 130.620, 145.033, 175.754。 【0 1 2 6】

[実施例15] Boc-TML1 g -Me1-OMe(22)

氷浴中のででEDC(0.52g, 2.70mmol)およびDMAP(0.988g, 8.10mmol)を無水DM(15mL)および無水DMF(5mL)中の21(0.531g, 1.35mmol)および20(0.863g, 2.70mmol)の混合物に加えた。窒素下、反応混合物を室温で一晩攪拌した後、真空濃縮した。残渣をDCM(75mL)に再溶解し、25mL 1N HClで3回洗浄した。有機層を無水硫酸マグネシウムで乾燥させ、濃縮し、シ 40リカゲルカラムクロマトグラフィー(酢酸エチル:ヘキサン = 7:3, v/v)により精製し、所望の生成物を得た(0.757g, 80.8%): 13 C NMR δ 20.120, 25.306, 28.294, 31.768, 35.42 7, 35.947, 36.669, 39.505, 40.311, 49.324, 51.959, 53.234, 53.453, 79.467, 112.0 95, 123.374, 125.169, 130.439, 132.856, 133.427, 136.666, 138.697, 145.091, 149.841, 156.081, 170.888, 172.298。

[0127]

[実施例16] $TML1_{\beta}$ —Me7—OMe TFA塩(23)

化合物22(0.757g, 1.09mmol)をDCM(5mL)およびTFA(2.5mL)中、室温で2時間攪拌した。この反応液を濃縮し、最少量のDCMに再溶解し、エーテルで沈殿させた。生成物を濾過により回収し、所望の生成物を得た(0.222g, 35.9%): 13 C NMR (CDCl₃ + CD₃ OD) δ 20:026, 25 50

.146, 31.738, 31.892, 35.271, 36.219, 39.163, 40.340, 49.006, 52.219, 53.396, 11 2.073, 123.260, 124.756, 130.377, 133.026, 133.180, 136.815, 138.595, 145.110, 1 49.283, 171.069, 171.619, 172.630

[0128]

[実施例17] PEG-cmc-TML1g-Me1-OMe(24)

窒素下、無水DCM(23mL)および無水DMF(6mL)中のPEG-cmc-Asp-Asp-OH(12, 1.6g, 0.0391mm ol)、23(0.277g, 0.391mmol)、EDC(0.076g, 0.391mmol)、およびDMAP(0.155g, 1.269mmol)の混合物を室温で一晩攪拌した。この溶液を真空濃縮し、残渣を130mL IPAで再結晶化させ、生成物を得た(1.543g, 92.5%)。UVアッセイで測定した生成物中のメルファラン量は2.86重量%であった: 13 C NMR & 19.642, 24.788, 31.175, 34.350, 35.975, 38.817, 39.9 10 05, 48.558, 51.553, 52.808, 60.897, 62.331, 65.145-72.878 (PEG), 111.394, 122.76 1, 124.425, 129.698, 132.105, 132.878, 135.804, 137.737, 144.316, 149.065, 160.4 32, 170.608, 171.598。

[0129]

[実施例18] Boc-TML1 β -AraC(25)

無水ピリジン(85mL)中のAra-C(1, 9.88g, 40.66mmol)の溶液を無水ピリジン(200mL)中の2 1(4.0g, 10.17mmol)、HOBT(5.49g, 40.66mmol)、EDC(15.61g, 81.32mmol)、およびNMM(8.93mL, 8.21g, 81.32mmol, 8当量)の混合物に加えた。窒素下、この反応混合物を40℃で48時間攪拌した後、真空濃縮した。残渣をDCM(300mL)に再溶解し、水(100mL)で3回、0.1N H Cl(100mL)で2回洗浄した。有機層を硫酸マグネシウムで乾燥させ、濃縮し、シリカゲルカ 20 ラムクロマトグラフィー(CHCl,:MeOH = 9:1, v/v)により精製し、所望の生成物を得た(3.26g, 52%):¹³C NMR δ 20.315, 25.560, 28.522, 31.660, 35.520, 36.200, 39.221, 50.239, 61.719, 75.171, 76.698, 79.635, 85.341, 88.052, 96.435, 122.894, 132.519, 1 33.190, 136.186, 138.007, 146.222, 149.109, 155.906, 162.191, 171.733。

[0130]

[実施例19] TML1β-AraC TFA塩(26)

化合物25(3g, 4.85mmol)をDM(15mL)に溶解した後、OCでTFA(7.5mL)を加えた。この反応混合物をOCで1.2時間攪拌し、冷水浴中で真空濃縮した。残渣をDCM-エーテルで沈殿させ、所望の生成物を得た(2.37g, 77%): 13 C NMR (CDCl $_3$ + CD $_3$ OD) $_{\delta}$ 20.0, 25.3, 31.5, 31.7, 35.0, 38.9, 50.2, 60.9, 75.1, 75.8, 85.7, 88.1, 94.9, 109.7, 113.5, 117.3, 12 30 1.1, 122.5, 132.6, 136.4, 138.4, 148.7, 149.5, 150.1, 159.2, 159.6, 160.1, 160.6, 161.1, 170.6, 172.7。

[0131]

[実施例20] <u>PEG-αmc-Asp-Asp-TML1β-AraC</u>、八量体(27)

化合物26および18を実施例18と同じ条件に付し、27を製造した。

[0132]

[実施例21] <u>化合物6aおよび6bのin vitroおよびin vivoデータ</u>

この実施例では、in vivoおよびin vitroデータを示し、非改変Ara-Cと比較している。 【0133】

<u>in vivo</u>

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胸腺欠損ヌードマウスにドナーマウスから採取したLX-1の4~5mm³組織片を皮下移植した。腫瘍トロカール部位を週2回観察し、触診できるものを週1回測定した。各マウスの腫瘍体積を測径器での二次元測定により調べ、式:腫瘍体積 = (長さ×幅²)/2により算出した。腫瘍が平均体積90mm³に達したときに、マウスを非改変Ara-CおよびPEG-Ara-C化合物からなるそれらの試験群に分けた。腫瘍サイズ分布が均等になるようマウスを分類し、4~6マウス/群に群分けし、永久識別のため耳にパンチ穴を開けた。薬物を毎分約0.5mLの速度で尾部静脈から静脈投与した、q3d×4(1、4、7および10日目)。化合物は、20mg/kgの等モルベース(絶対量の活性成分)で、またそれら各々のMTD(Ara-C、100mg/kg/投与量(毒性);6aおよび6b,40mg/kg/投与量(容量))に近い値でも与えた。マウス重量および腫瘍サイズは試験開始時と、第4週まで週2回測定した。薬物の有効性は処置したマウスと処置してい50

ない対照マウス(ビヒクルなし)との腫瘍増殖の比較により判定した。比較基準として5種類の指標を用いた: (a)28日目における平均腫瘍体積; (b)各腫瘍体積の試験開始時からの平均変化率; (c)対照群の腫瘍体積中央値が約800~1100mm³に達したとき(対数増殖期)に測定した腫瘍体積の相対率(%T/C); (d)21日目における(~2000mm³)腫瘍体積の相対率(%T/C)および(e)各群の腫瘍寛解(28日目の腫瘍体積が1日目と比べて小さくなっている)数。

[0134]

結果

化合物6bはわずか20%の活性親化合物量で非改変Ara-Cよりも優れた抗腫瘍活性を示した。また、化合物6aも有意な効果を示した。%T/Cは6bの値の約2倍であったが、それにもかかわらず、特に本発明の化合物がわずか20%の活性親化合物量で投与されたことを考慮すれば、それは非改変Ara-Cと比較して都合がよい。

【表 1】

化合物	t _{1/2} (h) ^a ラット血漿	1C ₅₀ (nM) " P388/0	LX-1 % T/C ^b
Ara-C	_	10	74.0 (100mg/kg)
化合物 6a	2. 1	123	122 (20mg/kg)
化合物 6b	53	958	59. 3 (20mg/kg)

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[0135]

[0136]

in vitroバイオアッセイ

P388/O(マウスリンパ系腫瘍, Southern Research Institute)細胞系を使用して一連のin vitroバイオアッセイを行い、非改変Ara-Cおよび化合物10のIC。を調べた。P388/O細胞を RPMI 1640培地(Whittaker Bioproducts, Walkersville, Maryland)+10%FBS(Hyclone Inc., Logan UT)で培養した。バイオアッセイは抗生物質およびファンギゾンを含有するそれ ら各々の培地で行った。

[0137]

Ara-CをDMSOに溶解し、培地で好適な濃度に希釈した。PEG-Ara-C化合物を水に溶解し、培地で好適な濃度に希釈した。

[0138]

アッセイを96ウェルマイクロタイター細胞培養プレートで2連で行った。

[0139]

化合物の2倍連続希釈をマイクロタイタープレートで行った。0.1%トリプシン/ベルセンを加えて37℃でインキュベートすることにより細胞を分離した。10%FBSを含有する各細胞系に好適な培地を加えてトリプシンを不活性化した。マイクロタイタープレートの各ウェルに10,000個の細胞を加えた。3日後、代謝性標識色素Alamar Blueを製造業者のプロトコールに従って添加し、細胞増殖を測定した。試験化合物および参照化合物のIC。値は上記に表で示している。

[0140]

本発明の好ましい実施形態であると現在考えられるものを記載してきたが、当業者ならば本発明の精神を逸脱しない限り、変形および改変をなしうることが分かるであろう。本発明の真の範囲にあるかかる変形および改変の全てを請求することを意図するものである。

^{*}全ての試験は2連、37℃で行い、t_{1/2}はPEG誘導体の消失により測定した。測定値の標準偏差 = ±10%。

[『]平均ベースライン腫瘍体積は1000mm』であった。

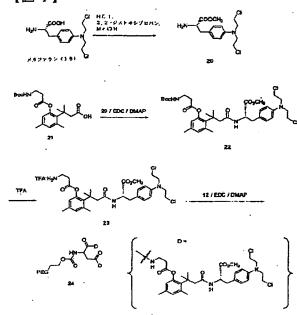
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【図面の簡単な説明】

[0141]

- 【図1】図1は、実施例1~6で記載する本発明の化合物を製造する方法の概略図である
- 【図2】図2は、実施例7~9で記載する本発明の化合物を製造する方法の概略図である
- 【図3】図3は、実施例10~13で記載する本発明の化合物を製造する方法の概略図である。
- 【図4】図4は、実施例14~17で記載する本発明の化合物を製造する方法の概略図である。
- 【図5】図5は、実施例18~20で記載する本発明の化合物を製造する方法の概略図である。

【図4】



【図5】

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TERMINALLY-BRANCHED POLYMETIC LINKERS AND POLYMETIC CONJUGATES CONTAINING THE SAME

TECHNICAL PIELD

The present invention relates to new types of terminally-activated polymeric materials which are useful in forming long-acting conjugates of bioactive materials. In particular, the invention relates to polymeric-based conjugates having increased therapeutic payloads and methods of preparing the same.

BACKGROUND OF THE INVENTION

Over the years, several methods of administering biologically-effective underials so memorals have been proposed. Many medicinal agents are available as water-soluble salts and can be included in pharmaceutical formulations relatively easily. Problems arise when the desired medicinal agent is either insoluble in aqueous fluids or is impidly degraded myivo. Alkaloids are often especially difficult to solubilize.

One way to solubilize medicinal agents is to include them as part of a soluble prodrug. Prodrugs include chemical derivatives of a biologically-active parent compound which, upon administration, eventually liberate the parent compound in vivo. Prodrugs allow the enterm to modify the caset and/or duration of oction of an agent in vivo and can modify the transportation, distribution or solubility of a drug in the body. Furthermore, prodrug formulations often reduce the toxicity and/or otherwise overcome difficulties encountered when administering pharmaceutical preparations. Typical examples of prodrugs include arganic phosphates or estens of alcohols or throalcohols. See Rendinguist-Planmaceutical Sciences, 16th Ed., A. Osol, Ed. (1980), the disclosure of which is incorporated by reference herein.

Produgs are often inalogically inert or substantially inactive forms of the parent or active compound. The rate of release of the active drug, i.e. the rate of hydrolysis, is

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influenced by several factors but espensilly by the type of bond joining the parent drug to the modifier. Care must be taken to avoid preparing prodrugs which are eliminated through the kidney or reticular endothelial system, etc. before a sufficient amount of hydrolysis of the parent compound occurs.

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Incorporating a polymer as part of a prodrug system has been suggested to increase the circulating life of a drug. However, it has been determined that when only one or two polymers of less than about 10,000 delions each or conjugated to certain biologically active substances such as alkaloid compounds, the resulting conjugates are rapidly eliminated in vivo, especially if a somewhat hydrolysis-resistant linkage is used. In fact, such conjugates are so impidly elegated from the body that oven if a hydrolysis-reme ester linkage is used, not enough of the perent molecule is regenerated in vivo to be the appearance.

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Camptothecin and related biologically active amilogs are often poorly water soluble and are examples of substances which would benefit from PEG prodrug technology. A brief overview of some previous work in the field is presented below.

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Ohya, et al., J. <u>Bioactive and Compatible Polyment</u> Val. 10 Jan., 1995, 51-66, disolose doxorubicin-PEG conjugates which are prepared by linking the two autoritinents via various linkages including esters. The molecular weight of the PEG used, however, is only about 5,000 at most. Thus, the <u>in vivo</u> benefits are not fully realized because the conjugates are substantially excreted prior to sufficient linkage bydrolysis.

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U.S. Patent No. 4,943,579 discloses certain simple 20(8)-camplotheein amino acid esters in their salt forms as water scalable prodrags. The reference does not, however, disclose using an amino acid as part of a linkage which would attaub the elixabid to a relatively high molecular weight polymer in order to form a prodrag. As evidenced by file data provided in Table 2 of the '579 petent, bydrolysis is rapid. Consequently, at physiologic pH, the insoluble base is rapidly generated after injection, binds to proteins and is quickly eliminated from the body before a therapeutic effect can be schieved. A related effort was directed to developing a water-soluble camputation sodium salt. Unfortunately, the water-soluble sodium salt of camputatheein remained too toxic for clinical application (Grattlieb et al., 1970 Center Elementer, Rep. 54, 461; Moortel et al., 1972 jbid, 56, 95; Gottlieb et al., 1972 lbid, 56, 103).

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Commonly-ossigned PCT publication WO96/23794 describes bis-conjugates in which one equivalent of the hydroxyl-containing drug is struched to each tenninal of the polymer. In spite of this advance, techniques which would further increase the payload of the polymer have been sought.

Thus, there continues to be a need to provide additional technologies for forming products of the capeutic moteties such as camptotheoin and related analogs. The present invention addresses this need.

SUMMARY OF THE INVENTION

In one aspect of the invention, compounds of Formula (1) are provided:

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$$R_1 = \begin{pmatrix} R_2 \\ C \\ R_3 \end{pmatrix} \begin{pmatrix} M \\ B \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix} \end{pmatrix} \begin{pmatrix} M$$

Whereir

R, is a polymeric residue,

Y, is O, 5 or NR4:

M is O, S or NR,;

(in) is zero or a positive integer, preferably 1 or 2;

(a) is zero or one;

E, is

 E_{24} are independently 11, $E_{\rm t}$ or



(n) and (p) are independently 0 or a positive integer;

 $Y_{z,j}$ are independently O, S or NR_{zz}

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 $R_{2:K}$ are independently selected from the group consisting of hydrogen, $C_{1:4}$ alkyls, $C_{3:4}$ branched alkyls, $C_{2:4}$ eveloalityls, $C_{1:4}$ substituted alkyls, $C_{3:4}$ substituted alkyls, expls, substituted styls, and $C_{1:4}$ betweenlyls, substituted $C_{1:4}$ between $C_{1:4}$ betweenlyls, substituted $C_{1:4$

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alkyls, $C_{i,\omega}$ alkozy, phenoxy and $C_{i,\omega}$ heleroalkozy,

 D_1 and D_2 are independently OH,

$$-1 - \begin{bmatrix} L_1 \\ L_2 \end{bmatrix}_1$$

$$R_{10} R_{15} Y_5$$

$$R_{14} R_{15} R_{16}$$

$$R_{13}$$

$$R_{13}$$

$$R_{14}$$

$$R_{15}$$

5 or additional branching groups described below.

Within formulae (IV) and (V), (v) and (i) are independently 0 or a positive integer up to about 6 and preferably about i;

I is NR₁₂ or

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 \mathbf{L}_1 and \mathbf{L}_2 are independently selected bifunctional linkers;

 $Y_{4,i}$ are independently selected from the group consisting of O, S and NR $_{\rm crit}$

 \mathbf{R}_{t+1} are independently selected from the group consisting of hydrogen,

C₁₋₄ alkyla, C₃₋₁₂ branchod alkyla, C₃₋₄ cyclonikyla, C₁₋₆ sub-nitoted alkyla, C₃₋₆ sub-stituted

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cycloalkyla, myła, mbstinued aryls, aralkyla, $C_{i,a}$ heteroalkyla, substituted $C_{i,a}$ hemoalkyla, $C_{i,a}$ alkoxy, phenoxy and $C_{i,a}$ heteroalkyla, $C_{i,a}$ alkoxy, phenoxy and $C_{i,a}$ heteroalkyla,

As is a moiety which when included in Formula (I) forms a multi-substituted aroundic hydrocarbon or a multi-substituted heterocyclic group; and

 B_1 and D_2 are independently selected from the group consisting of teaving groups, OB, residues of hydroxyl- or ambie-containing moioties.

In one particularly preferred aspect of the invention, the polymeric residue is also substituted on the dutial partian with a modety of formula (II) below:

(II)
$$E_{2} = \begin{bmatrix} E_{1} & Y_{1} & K_{2} \\ \vdots & \vdots & \vdots \\ E_{3} & E_{4} \end{bmatrix}$$

$$\begin{bmatrix} K_{2} & Y_{1} & K_{2} \\ \vdots & \vdots & \vdots \\ K_{3} & M \end{bmatrix}$$

where all variables are as previously defined. Bifunctional compounds are thus formed when the polymeric residue (R_c) includes both an alpha end an omega terminal linking group so that two, four or more equivalents of a biologically active agent, drug or protein, designated berein as R_t or B₂ cm be delivered. An example of such a bifunctional polymer transport form is illustrated below as formula (UI):

$$E_2$$
 E_3
 E_4
 E_5
 E_6
 E_6
 E_6
 E_6
 E_6
 E_6
 E_6
 E_6
 E_6
 E_6

wherein all variables are as described above.

For purposes of the present invention, the term "residue" shall be understood to mean that portion of a biologically active compound which remains after the biologically active compound has undergone a substitution reaction in which the product carrier portion has been attached.

For purposes of the present invention, the term "alkyi" shall be understood to include straight, branched, substituted, e.g., halo-, alkozy-, and mirro-, C., p alkyls, C., eyeloalkyis or aubstituted oyeloalkyis, etc.

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For purposes of the present invention, the term "substituted" shall be understrood to include adding or replacing one or more storks contained within a functional group or compound with one or more different atoms.

For purposes of the present invention, substituted alkyls include carboxyalkyls, aminoalleyls, dialkylsruines, hydroxyalkyls and mercaptealhyls; substituted cyclealkyls include moietics such as 6-chlorocyclohexyl; tryls include moietics such as napityl; substituted anyls include moietics such as 8-betorophenyl; aralkyls include moietics such as solary'; hoteroalkyls include moietics such as thylibiophene; substituted heteroalkyls tricked moietics such as 3-methoxy-thiophene; alkoxy includes moietics such as methoxy; and planoxy includes moietics such as 3-mirrophenoxy. Halo-ahall be understood to include Stuero, chloro, nodo and brome.

The term "sufficient encounts" for purposes of the present invention shall mean an annount which schieves a therapeutic effect as such effect is understood by those of ordinary skill in the srt.

One of the chief advantages of the compounds of the present invention is that the prodrugs have a higher payload per unit of polymer than previous techniques. It is generally preferred that the polymeric flast releases the trimethyl lock (TML) based prodrug intermediate by hydrolysis and than the resultant intermediate or "second prodrug" modely undergoes lastionization to regunerate, for extample, a moistly which is either a biologically active compound or a composition comprising a further prodrug. The high payload polymeric conjugates of the present invention are thus unique čelivery systems which can contain up to four or a greater number of notecoles of a drug.

Methods of making and using the compounds and conjugates described herein are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 · 5 schematically illustrate methods of forming compounds of the present invention; which are described in the Examples.

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DETAILED DESCRIPTION OF THE INVENTION

PORMULA (I)

In one preferred embodiment of the invention, there are provided compounds of

the formula:

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(I)

wherein;

R, is a polymeric residue;

Y, is O, S or NR.;

M is O, S or NR.;

(a) is zero us one;

(m) is zero or a positive integer,

E, is

En are independently II, E. or

(u) and (p) are independently 0 or a positive integer.

 $Y_{\rm po}$ are independently 0, S or NR,

 R_{240} are independently selected from the group consisting of hydrogen, C_{14} allayla, $C_{3,12}$ branched alkyla, C_{34} cycloalkyla, C_{14} substituted alkyla, C_{34} substituted

cyclosikyls, myłs, substituted myls, aratkyls, C_{14} beteroalkyls, substituted C_{14} beteroalkyls, C_{14} alkoxy, phenoxy and C_{14} heteroalkoxy;

D₁ and D₂ are independently OH,

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v) and (t) are independently 0 or a positive integer up to about 6 and preferably about i.

 \mathbf{L}_1 and \mathbf{L}_2 are independently selected bifunctional linkers;

 Y_{aa} are independently scheeted from the group consisting of O, S and NR $_{17}$

 $R_{\rm 13-17}$ are independently selected from the group consisting of hydrogen,

 $C_{1:4}$ alkyla, $C_{3:1}$ branched alkyls, $C_{3:4}$ cycloolkyls, $C_{1:6}$ substituted alkyls, $C_{3:6}$ substituted cycloalkyls, tryls, substituted aryls, tralkyls, $C_{1:6}$ bettroalkyls, substituted $C_{1:6}$ bettroalkyls, substituted $C_{1:6}$ bettroalkyls, $C_{1:6}$ bilency, phenoxy and $C_{1:6}$ bettroalkoxy:

At is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted hetemoryllic group; and

 B_{γ} and B_{γ} are preferribly independently selected from among leaving groups, OH,

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residues of hydroxyl-crutaining moieties or residues of amine-containing moieties.

In another preferred embudiment, D, and D2 are independently selected terminal

branching groups of formula (VI)

wherein

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 $E_{D,D}$ are selected from the same group which defines $H_{i,d}$ above, except that within the definition, D_i and D_i are charaged to D'_i and D'_i which are defined below. Writin this embadiment, D'_i and D'_i can be independently OH, a moviety of formula (IV) or (V), or

VII) —N—C—E4

wherein E_{Loop} are selected from the same group which defines E_{Loo} except that within the definition D_1 and D_2 are changed to D^n , and D^n , and D^n , independently OH_2 formula (IV) or formula (V). As can be appreciated from the above, when the terminal branching is taken to its fullest extent with a bifunctional polymer R_1 , up to sixteen (16) equivalents of drug can be loaded onto the polymeric platform.

In those aspects of this embodiment where his substituted polymeric residues are desired, some preferred polymeric transport systems of the invention are shown below as formula.

(III): $E_{2} = \begin{bmatrix} E_{1} & & & & \\ & & & \\ & & & \\ & & & \end{bmatrix} \underbrace{\begin{bmatrix} K_{2} \\ M \end{bmatrix}_{m}}_{R_{3}} \underbrace{\begin{bmatrix} K_{2} \\ M \end{bmatrix}_{m}}_{R_{3}} \underbrace{\begin{bmatrix} K_{1} \\ M \end{bmatrix}$

wherein all variables are as previously described.

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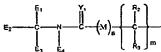
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The multi-loading polymer transport system of the present invention is based in large part on the polymeric residue designated horein as R_s. Optionally, R_s includes a capping group A. The polymer capping group A includes, for example, recicities such as hydrogen, CO₂H₃ C₃ elkyl mointies, and compounds of formula (II) shown below, which forms a bis-existen:

(m)



wherein, all variables are as previously described. It will be understood and approximated that the multiple terminal branching described above applies equally in the bis-systems as well.

With regard to the other variables which comprise the formulae of the present invention, the following are preferred:

Y to are each oxygen;

 $R_{2\text{-}10}$ and R_{12} are each preferably hydrogen or lower alley), e.g. C_{142}

 $R_{\rm 1H}\,R_{\rm 13}$ and $R_{\rm 14}$ are preferably -CH₆;

(m) is 1 or 2;

(n) and (p) are each either zero or an integer from 1-4;

(v) is zero or 1;

(t) is 1;

 L_1 is -(CH₂CH₂O)₂-; and

 L_3 is one of CH_2 , . $CH(CH_3)$, . $(CH_3)_2$, . $(CH_3)_3$ NH- , . CH_3 C(O)NHCH(CH_3), . $(CH_3)_3$ NH- , CH_3 C(O)NHCH $_2$, . $(CH_2)_2$ NH-C(O)(CH_3)_3NH- or $-CH_4$ C(O)NHCH(CH_3CH(CH_3)_2).

B. DESCRIPTION OF THE AT MOIETY

Referring to Formula (I), it can be seen that the Ar is a moisty, which when included in Formula (I), forms a multi-substituted aromatic hydrocarbon or a multi-substituted aromatic hydrocarbon or a multi-substituted heterucyclic group. A key feature is that the Ar moiety is aromatic in nature. Generally, to be errematic, the n electrons must be shared wifein a "cloud" both above and below the plane of a cyclic molecule. Furthermore, the number of a electrons must satisfy

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the Pitcket rule (4p+2). Those of ordinary skill will realize that a myriad of moirties will satisfy the aromatic requirement of the moiety and thus are suitable for use herein. One particularly preferred aromatic group is:

wherein $R_{\text{th},30}$ are selected from the some group which defines R_{th} . Alternative animatic groups include:

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wherein and Z_i and Z_i are independently CR_{22} or NR_{34} and Z_i is Q_i . S or NR_{34} where $R_{14,27}$ are selected from the same group as that which defines R_{11} or a cyano, nitro, carboxyl, anyl, substituted anyl or carboxyalkyl. Isomers of the five and aix-membered rings are also contemplated as well as beano- and dibrance-systems and their related congeners are also contemplated. It will also be appreciated by the arrisan of ordinary skill that the aromatic rings can optionally be substituted with interor-mores such as Q_i S, NR_{21} , etc. so long as Hückel's rule is obeyed. Furthermore, the aromatic or heterocyclic structures may optionally be substituted with halogen(s) and/or side chains as those terms are commandly tenderstood in the art. However, all offunctures aviable for Ar mointies of the present invention are capable of allowing the B_i or B_2 -containing moieties and the (R_{ij}) mainty to be in an ortho arrangement with the same plane.

C. DRUG GENERATION VIA HYDROLYSIS OF THE PRODRUG

The prodrug compounds of the present invention we designed so that the t_{t0} of hydrolysis is $< t_{t0}$ olimination in plasma.

The linkinges included in the compounds have hydrolysis rates in the plasma of the reasumal being treated which is short enough to allow sufficient amounts of the parent compounds, i.e. the arrino- or hydroxyl-containing bioactive compound, to be released prior to elimination. Some preferred compounds of the present invention have a t₁₀ for hydrolysis an plasma ranging from about 5 minutes to about 12 hours. Preferably, the compositions have a plasma t₁₀ bydrolysis ranging from about 0.5 to about 8 hours and most preferably from about 1 to about 6 hours.

D. SUBSTANTIALLY NON-ANTIGENIC POLYMERS

As stated above, R, is a water withhile polymeric residue which is preferably substantially non-entigenic such as a polyelkylene exide (PAO) or polyethylene glycol (PEG). In prefetred aspects of the inversion, R, further includes the provincely mentioned capping group, designated herein as A, which allows a bifunctional or bis-polymer system to be formed.

As an example, the PEG rotidue portion of the inventive compositions can be selected from the following non-limiting list:

-C(--Y₆)-(CH₂),-O-(CH₂CH₂O),-A,

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-C(=Y_a)- Y₁ -(CH_a)_PO-(CH_aCH_aO)_x-A,
-C(=Y_a)-NE₁₃-(CH_a)_PO-(CH_aCH_aO)_x-A,
-(CE_{3a}R₂₃)_x-O-(CH_aCH_aO)_x-A,
-(Nn₂₃-(CB₃)_x-O-(CH_aCH_aO)_x-A,
-(Nn₂₃-(CH_a)_x-O-(CH_aCH_aO)_x-A,
-(C₃-Y_a)-Y_x-(CH_a)_x-O-(CH_aCH_aO)_x-(CH_a)_x-Y_x-C(=Y_a)_x
-(C₃-Y_a)-Y_x-(CH_a)_x-O-(CH_aCH_aO)_x-(CH_a)_x-Y_x-C(=Y_a)_x
-(C₃-Y_a)-Y_x-(CH_a)_x-O-(CH_aCH_aO)_x-(CH_a)_x-NR₂₃-C(=Y_a)_x
-(CH_a)_x-O-(CH_a)_x-O-(CH_a)_x-O-(CH_a)_x-O-(CR_aX_ay)_x, and
-NR₂₃-(CH_a)_x-O-(CH_aCH_aO)_x-(CH_a)_x-NN₂₃-C(=Y_a)_x.

wherein Y_4 and Y_7 are independently $O_{\rm t}$ S or $NR_{\rm tot}$

z is the degree of polymerization:

 $R_{\gamma\beta}$, R_{γ} , and $R_{\alpha\beta}$ are independently selected from among H, $C_{\gamma\alpha}$ sikyls, $C_{\gamma\alpha\beta}$ branched alkyls, $C_{\gamma\alpha}$ eyeloolkyls, $C_{\gamma\alpha}$ substituted of $C_{\gamma\alpha}$ substituted ordinalkyls, $C_{\gamma\alpha}$ substituted ordinalkyls, $C_{\gamma\alpha}$ alkyls, $C_{\gamma\alpha}$ ordinalkyls, substituted $C_{\gamma\alpha}$ heteroalkyls, $C_{\gamma\alpha}$ ordinalkyls, phenoxy and $C_{\gamma\alpha}$ heteroalkoxy;

e and f are independently zero, one or two; and

A is a capping group.

The degree of polymerization for the polymer (x) can be from about 10 to about 2,300. This represents the number of repeating units in the polymer chain and is dependent on the molecular weight of the polymer. The (A) moiety is a capping group as defined herein, i.e. a group which is found on the terminal of the polymer and, in some aspects, can be selected from any of H, NH₂, OH, CO₂H, C₁₄ alkyls or other PiG terminal activating groups, as such groups are understood by those of ordinary skill.

Also useful are polypropylene glycols, branched PEG derivatives such as those described in commonly-assigned U.S. Patent No. 5,643,575, "atar-PEG's" and multi-armed PEG's such as those described in Shearwater Polymers, Inc. catalog "Polyethytene Glycol Derivatives 1997-1998". The disclosure of each of the foregoing is incorporated berein by reference. It will be understood that the water-soluble polymer can be functionalized for attachment to the bifunctional linkage groups if required without undue experimentation.

 b_1 is further embodiment R_1 is optimally selected from among one or more of dextran, polyvinyl aloohols, carbohydrate-based polymers, hydroxynropylmethactyl-

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armide, polyalkylene oxides, and/or comolymers thereof. See also commonly-assigned U.S. Paient No. 6,153,655, the contents of which are incorporated herein by reference.

In many aspects of the greatest invention, bis-activated polyethylene glycols are preferred when di-or more substanted polyether conjugates are desired. Aftermitively, polyethylene glycols (PEG's), mano-activated, C_{1.4} sikyl-terminated polyalkylene oxides (PAO's) such as mano-methyl-terminated polyethylene glycols (mPEG's) are preferred when mano-substituted polyethylene glycols (mPEG's) are preferred when mano-substituted polyethylene glycols (mPEG's) are preferred.

In order to provide the desired hydrolyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids can be used as well as mono- or di-PIG arrines and mono- or di-PEG dials. Suitable PAO acids can be synthesized by first curverling mPEG-OH to an ethyl esser followed by saponification. See also Gehrhardl, H., et al., Polymer: Builetin 18: 467 (1987) and Veronese, P.M., et al., J. Controlled Release 10: 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mPEG-OH into a 1-banyl ester followed by acid cleavage. See, for example, community assigned U.S. Pelent No. 5,605,976. The disclosures of each of the foregoing are macaporated by reference berein.

Although PAO's and PEO's can very substantially in average molecular weight, the polymer portion of the prodrug is at least about 20,000 weight average in most aspects of the invention. Preferably, R_c has a weight average molecular weight of from about 20,000 to about 100,000 and more preferably from about 25,000 to about 60,000. The average molecular weight of the polymer selected for inclusion in the prodrug must be sufficient to us to provide sufficient circulation of the prodrug before hydrolysis of the linker.

The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers turbade polyalkylene exide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyex yethylenated polyols, expolymers thereof end block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

As an alternative to PAO-based polymers, effectively non-antigenic materials such as dexiran, polyvinyl alochols, carbohydrate-based polymers, hydroxypropylmethacrylamido (HPMA), and copolymers thereof etc. and the like can be used if the same type of activation is employed as described herein for PAO's such as

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PEG. Those of ordinary skill in the art will realize that the foregoing list is morely illustrative and that all polymeric materials having the qualities described herein are contemplated. For purposes of the present invention, "effectively nun-antigenic" and "substantially non-antigenic" shall be understood to include all polymeric materials understood in the art as being substantially non-toxic and not eliciting an appreciable immune response in manumals.

It will be clear from the foregoing that other polyalkylene oxide derivatives of the foregoing, such as the polypropylene glycol acids, etc., as well as other bi-functional linking groups are also contemplated.

E. PRODRUG CANDIDATES

1. Residues of Hydroxyl-containing Compounds

Commonthecin and Related Topoisumerase I Inhibitors

Cemplothecin is a weter-insoluble cytotoxic alkaloid produced by Compactness accuminate trees indigenous to Chris and nothapodytes foetida rrocs indigenous to India, Comptothecin and related compounds and analogs are also known to be potential anticamor or autitumor agents and have been shown to exhibit these activities jn vitro and jn vivo. Comptothecin and related compounds are also candidates for conversion to the prodrugs of the present invention.

Camptothocis and certain related analogues share the structure:

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From this core structure, several known analoga have been prepared. For example, the A ring in either or both of the 10- and 11-positions can be substituted with an OH. The A ring can also be substituted in the 9-position with a straight or branched C_{k+10} alkey, optionally linked to the ring by a heterostom i.e. O or S. The B ring can be substituted in the 7-position with a straight or branched C_{k+10} alkyl or substituted alkyl-, C_{k+10} cycloakyl, C_{k+10} alkoy, phenyl alkyl, etc., alkyl carbonate, alkyl

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carbandes, phenyl hydracine derivatives, amino-, aminoalkyl-, uralkyl, etc. Other substitutions are possible in the C, D and H rings. See, for example, U.S. Potent Nos. 5,004,758; 4,943,579; Re 32,518, the contents of which are incorporated herein by reference. Such derivatives can be made using known synthetic techniques without urable experimentation. Per ferred comptohecin derivatives for use bevein include those which include a 20-OH or another OH motety which is capable of reacting directly with activated forms of the polymer transport systems described herein or to the linking motety intermediates, e.g. immodiatestic ucid, etc., which are then attached to a polymer such as PFG.

Reference to comptother mailogs becein has been made for purposes of illustration and not limitation.

b. Ingenes and Pactitaxel Derivatives

One class of compounds included in the procing compositions of the present invention is taxanes. For purposes of the present invention, the term "taxane" includes all compounds within the manne family of terpence. Thus, taxol (paciliaxel), 3'substituted ign-butoxy-outboryl-amine derivatives (taxoneres) and the bike as well as other mailogs which are readily synthesized using standard organic techniques or are available from commercial sources such as Sigmo Chermael of St. Louis, Missouri are within the scope of the present invention. These decivatives have been found to be offective anti-cancer agents. Numerous studies indicate that the agents have activity against several malignancies. To dote, their use has been severely limited by, among other things, their short amply, poor water solubility and a tendency to cause hypersensitivity. It is to be understood that other taxanes including the 7-aryl-carbamates and 7-carbarates disclosed in commany assigned U.S. Patent Nos. 5,622,986 and 5,547,981 can also be included in the produtage of the present invention. The contents of the foregoing U.S. patents are incorporated herein by reference. Paclitusel is a preferred tenance.

e. Additional Biologically-Active Moistics.

In addition to the foregoing molecules, the prodrug formulations of the present invention can be prepared using many other compounds. For example, biologically-active compounds such as his-PEG conjugates derived from compounds such as

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poduphyllotoxin

triozole-based antifungal agents such as fluconazole:

ar eiolopicos

OT AIR-C:

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The parent compounds selected for prodrug forms need not be substantially water-insoluble, although the polyroer-based prodrugs of the present invention are especially well suited for delivering such water-insoluble compounds. Other useful parent orangoninds include, for example, certain low molecular weight biologically active proteins, enzymes and peptides, including peptide glycans, as well as other anti-minor agents; condivorserular agents such as forskolin; anti-moplistics such as condiretestatin, viriblastine, devotublein, maytansine, etc.: anti-infectives such as vencentysin, crythromycin, etc.: anti-fungals such as mystatin, amphotoricin B, triazoles, pepulucandins, pineumocandins, echinocandins, polyexins, nildconycins, pradimicins, betanomicins, etc. see, "Artibiotics That Inhibit Pungal Cell Wall Development" Annua, Ret. Microbiol. 1994, 48:471-97, the contents of which are incorporated bereau by reference; anti-arciety agents, gast-vinestinal agents, control nervous system-activating agents, unalgences, fertility or contraceptive agents, anti-inflammatory agents, secroidal agents and the like.

The foregoing is illustrative of the biologically active moietics which are anitable for the prodruga of the present invention. It is to be understood that those biologically active materials not specifically mentioned but having suitable ester-forming groups, i.e. hydroxyl moistics, are also intended and are within the scope of the present invention may also include minor amounts of compounds contingates of the present invention may also include minor amounts of compounds containing not only one equivalent of drug and polymer but also a minety which does not effect bioactivity in vivo. For example, it has been found that in some instances, in spite of reacting diacids with drug molecules having a single linkage point, the reaction conditions do not provide quantitative amounts of products with two equivalents of drug per polymer. By-products of the reactants can sometimes be formed auch as any largest floarbodilimides are used.

2. Residues of Amine-containing Compounds

In some aspects of the invention, B, or B, is a residue of an arome-containing compound, a non-liniting list of such smitchle compounds include residues of organic compounds, enzymes, proteins, polypeptides, etc. Organic compounds include, without limitation, moteries such as anthracycline compounds including demorables, do down obtaining permission, metalline masterd, metallas, Ara-C (cytosine archinoside) and

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related anti-metabolite compounds, e.g., gemeitabine, etc. Alternatively, B can be a residuo of un amino-containing cardiovascular ageni, anti-aeoplastic, anti-infectiva, antiflunged such as mystatus and amphotoricin B, anti-anxiety agent, gastrointestinal agent, central nervous system-activating agent, analyssic, fertility agent, contraceptive agent, anti-inflammatory agent, steroidal agent, anti-urcomic agent, vasodilating agent, vasoconstricting agent, etc.

in a preferred aspect of the invention, the amino-containing compound is a biologically active compound that is suitable for medicinal or diagnostic use in the treatment of animals, e.g., mammals, including humans, for conditions for which such treatment is desired. The foregoing list is meant to be illustrative and not limiting for the compounds which can be modified. Those of ordinary skill will realize that other such compounds can be similarly modified without undue experimentation. It is to be understood that those biologically active materials not specifically mentioned but having suitable amino-groups are also intended and are within the scape of the present invention.

The only limitations on the types of amhor-containing molecules suitable for inclusion herein is that there is available at least one (primary or secondary) sminecontaining position which can react and link with a carrier portion and that there is not substantial loss of bioactivity after the product system releases and regenerates the percut commound.

It is noted that parent compounds suitable for incorporation into the prodrug compositions of the invention, may themselves be substances/compounds which are not active after hydrolytic release from the linked composition, but which will become active ofter undergoing a further chemical process/reaction. For example, an assissmoor drug that is delivered to the bloodstream by the double prodrug transport system, may remain inactive until entering a cancer or tumor cell, whereupon it is notivoted by the cancer or tumm cell chemistry, e.g., by un

enzymetic reaction unique to that cell.

3. Leaving Groups

In those aspects where B_{α} or B_{α} is a leaving group, smithle leaving groups include, without limitations, moieties such as N-hydroxybenzutriazolyl, halugen, N-bydroxyphthalimidyl, p-nitrophonoxy, imidazolyl, N-bydroxysuccinimidyl; this soliding thione, or other good leaving groups as will be apparent to those of ordinary

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skill. The synthesis reactions used and dracribed herein will be understood by those of ordinary skill without under experimentation.

For example, an anylated intermediate of compound (I) can be reacted with a reacted such as 4-citrophenyl chloroformate, disaccinimidyl carbonate (DSC), carbonyldiimidazole, (hiszolidine thione, etc. to provide the desired activated derivative.

The selective anylation of the phenotic or entilinic portion of the p-hydroxybernyl alcohol or the p-aminobernyl alcohol and the o-hydroxbernyl alcohol or the o-aminobernyl alcohol or the carried out with, for example, this colliding thione activated polymers, secentimidyl carbonate activated polymers, curron yite acid activated polymers, blocked amino acid derivatives. Once in place, the "activated" form of the PEG prodrug (or blocked prodrug) is ready for conjugation with an amino- or hydroxyl-containing compound.

F. SYNTHESIS OF THE POLYMERIC PRODRUG TRANSPORT SYSTEM

Synthesis of representative polymer prodrugs is set forth in the Numples. Generally, however, in one preferred method of preparing the product transport systems, the polymer residue is first attached to the branching groups. Separately, the biologically active reciety or drug, e.g. Drug-OH or Drug-NH₃ (B₁ or B₂ of formula I) is attached to the TML component which may also include a bifunctional spacer thereon at point of attachment to the polymer. Next, the polymeric residue combining the terminal branches is reacted with the drug-TML portion under conditions sufficient to form the final product.

Attachment of the bifunctional spacer containing the TMI.-Drug component to the polymer partion is preferably carried out in the presence of a coupling agent. A nunliming list of suitable coupling agents include 1,3-discopropy/carbodiimide (DIPC), any suitable diality) carbodiimides, 2-tholo-1-alityl-pyridinium halides, (Mukaiyamo reagents), 1-(3-dimethylaminopropyl-3-ethyl carbodiimide (EDC), propane phosphonic acid cyclic unbydride (PPACA) and piceryl dichlorophos-photes, etc. which are available, for example from commercial sources such as Sigma-Aldrich Chemical, or synthesized using known techniques.

Preferably the substituents are reacted in an inert solvent such as methylene chloride, chloroform, DMF or mixtures thereof. The reaction also preferably is conducted

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in the presence of a base, such as directly leminopyridine, dilsopropylethylemina, pyridine, triedrylamine, etc. to neutralize any soids generated and at a temperature from orC up to about 22°C (room tempersture).

More particularly, one method of preparing a polymeric transport system includes reacting a compound of the formula (VIII):

wherein all variables are as previously defined and

(IX)

B', is a residue of a hydroxyl- or an amine-containing moiety; with a compound of the formula (IX):

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$$R_1 = \begin{bmatrix} R_2 \\ C \\ R_3 \end{bmatrix}_m \begin{bmatrix} Y_1 \\ Y_2 \\ Y_3 \end{bmatrix}_{E_3} \begin{bmatrix} E_6 \\ E_7 \end{bmatrix}$$

wherein R_i is a polymeric residue; Y_i is O, S or NR_e M is O, S or NR_e (a) is zero or one; (m) is 0 or a positive integer, Y_{23} are independently O, S or NR $_{101}$ and R_{23} are independently selected from the group consisting of hydrogen, C_{10} alkyls, C_{20} branched alkyls, C_{14} cycloalkyls, C_{14} substituted alkyls, C_{24} substituted cycloalkyls, aryla, substituted aryls, smalkyls, $C_{1,4}$ beteroaikyls, substituted $C_{1,4}$ beteroalkyls, $C_{1,4}$ alkoxy, phenoxy and $C_{i,k}$ heremalkoxy;

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$$E_a$$
 is $-\left(\begin{bmatrix} 1\\1\\0\\0\\0\end{bmatrix}^{\frac{n}{2}} C - O_3\right)$

E.a are independently H. E., or

wherein D, and D, are independently OH or a leaving group which is capable of reacting with an emprotected amine or hydroxyl or a terminal branching group;

(n) and (p) are independently 0 or a positive integer;

 $Y_{\mathfrak{p},\mathfrak{p}}$ are independently $O_{\mathfrak{p}}S$ or $NR_{\mathfrak{m}\mathfrak{p}}$ and

 $R_{\star,to}$ are independently selected from the group consisting of hydrogen, $C_{1,4}$ alkyls, $C_{3,4}$ branched alkyls, $C_{3,4}$ cycloalkyls, $C_{1,4}$ substituted alkyls, $C_{3,4}$ substituted cyclonikyls, aryls, substituted aryls, aralkyls, $C_{1,4}$ heteroalkyls, substituted $C_{1,4}$ heteroalkyls, $C_{1,4}$ alkaxy, phenoxy and $C_{1,4}$ heteroalkoxy.

in further espects of the method, D_s and D_s are independently selected terminal branching groups of formula (X)

where E_{1548} are selected from the same group which defines $E_{1,38}$ except that D_1 and D_4 are changed to D_2 and D_4 which are defined below. Within this embodiment, D_3 and D_4 can be independently OH_1 a moiety of formula (IV) or (V), or OH_2)

wherein $F_{qk,20}$ are selected from the same group which defines $E_{q^*p_*}$ except that D_q and D_q are changed to D^{**}_q , and D^{**}_q which are defined as being independently OII or a leaving group which is capable of reacting with an unprotected amine or hydroxyl.

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Such synthetic techniques allow up to sixueen (16) equivalents of carboxylic acid or activated carboxylic acid, for example, to be attached. As shown in the preferred structures herein, PEG residues with terminally branched untiti-acids are preferred aspects of the invention.

Regardless of the synthesis selected, some of the preferred compounds which result from the synthesis techniques described herein include:

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where B is a residue of an amine or a hydroxyl- containing drug.

In another preferred aspect of the invention, the compounds of the present invention are of formula (XII):

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wherein all variables are as previously defined above.

G. IN VIVO DIAGNOSTICS

A further aspect of the invention provides the conjugator of the invention optionally prepared with a diagnostic tag linked to the transport enhancer described above, wherein the tag is selected for diagnostic or imaging purposes. Thus, a suitable tag is propered by linking any suitable moiety, $Q_{i,k,l}$, an amino acid recidue, to any ort-standard emitting isotope, radio-opaque label, magnetic resonance label, or other num-radioactive isotopic labels suitable for magnetic resonance imaging, fluorescence-type labels, labels exhibiting visible colors and/or capable of fluoresceing under ultraviolet, influred or electrochemical stimulation, to allow for imaging tumor tissue during surgical procedures, and so forth. Optionally, the diagnostic tag is incorporated into and/or linked to a conjugated therspectate moiety, allowing for monaturing of the distribution of a therapeutic biologically active material within an animal or human patient.

In a still further aspect of the inversion, the inventive tagged conjugates are readily prepared, by art-known methods, with any anitable label, including, 6.2. radioisotope labels. Simply by way of example, these include "Hottine, "Godine, "Fertretium and/or "Hindium to produce radioinmumoscintigraphic agents for selective uptake into turnor ceils, in vivo For instance, there are a number of art-known methods of Ending peptide to Te-99m, including, simply by way of example, those shown by U.S. Patent Nos. 5,328,679; 5,888,474; 5,997,844; and 5,997,845, incorporated by reference berein.

Broadly, for anatomical localization of namor tissue in a patient, the conjugate tag is administered to a patient or animal suspected of having a tumor. After sufficient time to allow the labeled immunoglobulin to localize at the turner site(s), the signal generated by the label is detected, for instance, visually, by X-ray radiography, computerized transocial tontography, MRI, by instrumental detection of a luminescent tag, by a photo semming device such as a garman camera, or any other method or instrument appropriate for the nature of the selected tag.

The detected signal is then converted to un image or anatomical and/or physiological determination of the turnor site. The image makes it possible to locate the turnor in vivo and to devise an appropriate therupcutic strategy. In those embodiments

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where the tagged mainty is itself a therapeutic agents, the detected aignal provides evidence of anatomical localization during treatment, providing a baselino for follow-up diagnostic and therapeutic interventions.

11. METHODS OF TREATMENT

Another aspect of the present invention provides methods of treatment for various modical conditions in mammals. The methods include administering to the mammal in need of such treatment, an effective amount of a prodrug, such as a multi-loaded Ara-C-PEG conjugates, which has been prepared as described herein. The compositions are useful for, among other things, treating neoplastic disease, reducing humor hunden, preventing metastasts of neoplasms and preventing recurrences of tumor/moplastic growths in mammals.

The amount of the prodrug administered will depend upon the parent molecule included therein. Generally, the amount of prodrug used in the treatment methods is that amount which effectively achieves the desired therapeutic result in mammats. Naturally, the desiges of the various prodrug compounds will vary somewhat depending upon the parent compound, rate of in vivo hydrolysis, molecular weight of the polymer, etc. In general, however, prodrug toxanes are administered in amounts ranging from about 5 to about 500 mg/m² per day, head on the amount of the taxane moiety. Camputchedin prodrugs are also administered in amounts ranging from about 50 mg/m² per day. The range set forth above is illustrative and those skilled in the art will determine the optimal dusting of the prodrug selected besed on clinical experience and the treatment indication. Actual desages will be opparent to the artisan without undue experimentation.

The prodrugs of the present invention can be included in one or more autable pharmaceutical compositions for administration to mammals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral aud/or parenteral routes depending upon the needs of the artisun. A solution and/or suspension of the composition may be utilized, for example, as a currier vehicle for injection or infiltration of the composition by any art known methods, e.g., by intravenous, intramuscular, subdermal injection and the like.

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Such administration may also be by influsion into a body space or cavity, as well as by inhalation and/or intranssal routes. In preferred aspects of the invention, however, the prodrugs are parenterally administered to maximals in need thereof.

1 EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective stope of the invention. The underlined and boild-faced numbers recited in the Examples correspond to those shown in Figures 1-5.

General. All reactions were run under an atmosphere of dry mirogen or argon.

Commercial resignts were used without further purification. All FEG compounds were dried under vacuum or by amotropic distillation (tolurne) prior to use. Hi spectra were obtained with a JEOL FT NMR System JNM (SIX 270 instrument using described from as solvent unless specified. "C NMR spectra were obtained at 67.80 MRz on the JNM GSX-276, Chemical shirls (6) are reported in parts per million (ppm) downfield from tetramethylsiano (TMS) and coupling constants (J values) are given in herro. (H2). All FEG conjugated compounds were dissolved (~15 mg/mL) in sterile saline (0.9%) for injection prior to be vivo drug greatments and were given as their ara-C equivalents (absolute amount of ora-C given).

HFIA.7 Method. Analytical HFLC's were performed using a CB reversed phase column (Beckman, ultrasphere) under isocratic conditions with an 80:20 mixture (wh) of methanol-water as mobile phase. Peak elutions were manitored at 254 mi using a 1!V detector. To detect the presence of any free PEO and also to confirm the presence of PEGYLA/FiD product, an evaporative light scattering detector (ELSD), Model FL-EMD 950 (Polymer Laboratories), was employed. Based on ELSD and UV analysis, all the final PEGylasted products were free of native drug and were a 95% pure by HPLC. Analysis of Ara-C Content in PEG Derivatives. For the determination of the ara-C content in PEG derivatives, M-acctyleytidine was used as a model. The UV absorbance of M-acctyleytidine in H₂O was determined at 257 mm for six different concentrations ranging from 0.01 annolmal, to 0.05 annolmal. From the standard plot of absorbance vs. concentration, the absorption coefficient, a. of M-acctyleytidine was calculated to be 36.4 (O.D. et 257 nm for 1 mg/mL with 1.0 cm tight path). PEGylated ara-C derivatives were

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40 kHa) and the UV absorbance of these compounds at 257 nm was determined. Using this value and employing the absorption coefficient, c, obtained from the above, the concentration of ara-C in the sample was determined. Dividing this value by the sample concentration provided the percentage of ara-C in the sample. Analysis of Mclphalan Content in PEG Derivatives. For the determination of the melphalau content in PEO derivatives, melphalan was used as a standard. The UV absortance of melphalan in DMF-H₂O (9:1, v/v) was determined at 264 nm for five different concentrations ranging from 0.02 $\mu mol/ml$, to 0.06 $\mu mol/ml$. From the standard plot of absorbance is, concentration, the absorption coefficient, a, of melphalm was calculated to be 54.6 (O.D. at 264 nm for 1 mg/ml. with 1.0 cm light path). PEGYLATED melphalm derivatives were dissolved in DMF-H₂O (9:1, v/v) at an approximate consentration of 0.013 panel/mL (based on a MW of 40 kDa) and the UV absorbance of these compounds at 264 rm was determined. Using this value and employing the absorption coefficient, s, obtained from the above, the concentration of melphalan in the sample was determined. Dividing this value by the sample concentration provided the percentage of melphalm in the sample. Abbreviations. DCM (dichloromethane), DMAP (4-(dimethylamino)pyridine), EDC (1ethyl-3-(3-dimethylaminopropyl)carbodiimide), HOBT (1-hydroxybenzotriazole), IPA (2proposed), NMM (N-methylmorpholine), TFA (trifluoroacetic acid).

dissolved in H₂O at an approximate concentration of 0.015 µmot/ml. (based on a MW of

proposol), NMM (A-methylmorpholine), TFA (trifluoreacette acid).

Example 1.

Compound 3a. A mixture of sra-C (1, 1.73 g, 7.12 mmol), 2a (700 mg, 1.78 mmol),

HOBT (0.96 g, 7.12 mmol), and EDC-HCI (2.73 g, 14.25 mmol) in anhydrous pyridine
(50 mL) was suirred at room temperature for 2 b, the temperature raised to 40 °C and the
reaction continued overnight. The solvent was temoved, methylme chloride (50 mL) was
used to dissolve the mixture followed by washing with water (3 × 30 mL) and then with

0.1 N HCI (2 × 30 mL). The organic layer was dried over anhydrous MgsCQ, and the
solvent removed in vacuo to give the drude product which was purified by silics gol

column chromatography (5 to 10% MeCH in DCM) to give 638.6 mg (5274) of 3a os a

white solid: "H MMR & 1.42, 1.55, 2.17, 2.26, 2.46, 2.79, 3.84, 3.91, 4.14, 4.33, 4.53, 5.49, 6.07, 6.17, 6.52, 6.76, 7.31, 7.67, 8.16, 8.62; "C NMR & 17.77, 20.11, 25.36, 28.32, 31.51, 31.96, 39.57, 50.18, 50.45, 61.88, 74.50, 80.15, 85.90, 88.58, 96.25, 122.51,

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132.82, 133.34, 136.73, 138.22, 146.57, 149.90, 155.65, 155.96, 162.08, 171.89, 174.06. Example 2.

Compound 3b. Compound 1 was coupled with 2b using a similar condition as in Example 1 to produce 3b in 54% yield: ¹¹C NMR 6_17.23, 17.92, 18.33, 25.49, 26.32, 31.51, 31.58, 31.99, 32.46, 39.52, 40.09, 50.08, 50.22, 61.72, 74.50, 74.94, 50.11, 80.15, 85.45, 55.90, 88.01, 88.38, 96.25, 122.51, 128.77, 129.03, 129.16, 131.66, 132.82, 136.24, 136.73, 138.22, 146.05, 146.57, 149.90, 155.65, 155.96, 171.85, 171.89, 174.06. Example 3.

Compound 4a. Compound 3a (638.8 mg, 1.03 menol) was stirred in anhydrous DCM (6

10 mL) and TFA (4 mL) at room temperature for 2 h. Ethyl other was added to the solution
to precipitate the crude product which was filtered and washed with ether to give 4a so a
white solid (534.5 mg, 52%): "IN NMR (DMSO-4a) 5 1.52 (a, 311, (CH₃),CH) 1.55 (s, 3H,
(CH₃),CH), 1.62 (d, 1 H, J = 8.1 H₂ (CH₃),CH), 2.22 (s, 3H, CH₃Ar), 2.57 (s, 3H,
CH₃Ar), 2.97 (a, 2H, CH₂C(=O)), 3.41-4.27 (m, 5 H, sm-C's H-2'-H5'), 6.09 (d, 1H,
J = 5.4, sm-C's H-1'), 6.67 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.12 (d, J = 5.4, H-6), 8.05
(d, J = 8.1, H-5), 8.67 (hs, 1H, TFA): "NC NMR (DMSO-4) 5 15.45, 19.67, 24.97, 31.05

(d, J=8.1, H-5), 8.67 (bs, 1H, TFA); ¹²C NMR (DMSO-4), 8 15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.68, 171.87.

20 Example 4.

Compound 4b. Compound 3b was subjected to the same condition as in Example 3 to give 4b in 82% yield: 'H NMR (DMSO-d_d) 5_1.52 (a, 3H, (CH₂)₂CH) 1.55 (a, 3H, (CH₂)₂CH). 1.62 (d, 1 H, J = 8.1 Hz, (CH₂)₂CH), 2.22 (a, 3H, CH₂Ar), 2.57 (a, 3H, CH₂Ar), 2.97 (a, 2H, CH₂C(=0)), 3.41-4.27 (m, 5 H, ara-C's H-2'-H5'), 6.09 (d, 1H, CH₂Ar), 2.97 (a, 2H, CH₂C(=0)), 3.41-4.27 (m, 5 H, ara-C's H-2'-H5'), 6.09 (d, 1H, Ara-C's H-2'-H5'), 6.09 (d, 1H, CH₂C(=0)), 3.41-4.27 (m, 5 H, ara-C's H-2'-H5'), 6.09 (d, 1H, CH₂C(=0)), 6.00 (

J = 5.4. am-C*s H-1*), 6.67 (s, 111, Ac-11), 6.90 (s, 111, Ar-11), 7.12 (c, J = 5.4, H-6), 8.05 (d, J = 5.1, 11-5), 8.67 (bs, 111, TFA); ¹³C NMR (DMSO-d_c) 8_15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 83.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 156.20, 158.72, 162.02, 169.68, 171.87.

30 Example 5.

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Companied 6a. A mixture of PEG-aspartic acid (mev. 40,000, 5, 3 g, 0.074 mmol), 4a (285.6 mg, 0.74 mmol), NMM (240 mg, 2.38 mmol), HOBT (120.5 mg, 0.69 mmol), and

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EDC-HCI (228.4 mg, 1.19 mmol) in anhydrous DCM (50 mL) was stirred at 0 °C for 30 minutes. The reaction was allowed to warm to room temperature and continued for 3 days and filtered. The filtrate was concentrated in source and the residue recrystallized from IPA to give 2.7 g (90%) of product. The anastati of arti-C in the product measured by UV assay was 2.11 wt96: "C NMR 5 14.40, 19.22, 24.56, 31.17, 38.26, 58.90, 47.94, 48.67, 49.66, 60.17, 61.12, 61.90, 67.86-70.87 (PES), 71.70, 74.50, 85.01, 87.53, 95.28, 121.39, 131.18, 132.68, 133.19, 134.77, 137.70, 145.26, 138.93, 155.23, 160.12, 161.56, 168.39, 170.72, 170.92, 171.27, 171.34.

Raample 6.

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2¢

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Compound 6b. Compound 4b was subjected to the same condition as in Example 5 to give 6b in 88% yield. The amount of gra-C in the product measured by UV assay was 1.68 wt%: "C NMR 6 15.12, 16.22, 24.52, 24.73, 29.55, 30.55, 31.15, 38.04, 38.59, 47.66, 49.16, 49.93, 50.18, 60.93, 61.12, 62.90, 69.44-71.59 (PEG), 71.70, 74.50, 84.78, 84.90, 87.53, 94.85, 127.60, 130.20, 135.51, 136.10, 141.70, 145.15, 147.50, 155.00, 161.20, 169.47, 170.62, 170.92, 171.27.

Example 7.

Compared 9. PEG diel (7, 55 g. 1.38 renol) was azentroped in toinene over a 2 hour period followed by removal of 200 mL of solvent by rotary evaporation. The solution was cooled to ~30 °C and triphosgene (0.544 g. 1.83 runol) was added as solid followed by unhydrous pyridine (0.434 g. 5.49 mmol), and the reaction mixfure stirred of 50 °C for 1 hour. N-hydroxyphthalamide (8, 1.12 g. 6.88 mmol) and anhydrous pyridine (0.54 g. 6.88 mmol) were added to the chloroformate mixture and the reaction stirred for a furface 2 hours at 50 °C then for 12 hours at room temperature. The reaction mixture was filtered through filter paper and the solvent removed in vacuo and the product crystallized from methylene chloride-ethyl ether (1100 mL, \$12, vlv) to give the product (50.9 g. 92%): "C NMR 6 123.62, 128.10, 134.55. 152.00, 160.00.

Example 8.

PEO-care-Asp-O-4-Bu (11). Compound 9 (raw. 40,000, 20 g, 0.459 mmol) and aspartic avid di r-butyl ester IICl (10, 1.0 g, 3.55 mmol) were dissolved in anhydrous DCM, followed by addition of DMAP (0.433 g, 3.55 mmol). The solution was refuxed overnight followed by precipitation by addition of ethyl efter (1 L). The solid was isolated by fibration and recrystallized from IPA (1 L) twice. The filter cake was washed

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with IPA (200 mL) and ether (200 mL) to give 15.6 g (78%) of product after drying at 45 °C in vacuo: "C NMR 8 27.837 (CH₂CO₂C(CH₃)₃), 27.991 (CH(CO₂C(CH₃)₃), 37.752 (CECH,CO.), 50.800 (NHCII), 64.212 (OCH,CH,OC(=O)NH), 81.333 (CH₂CO₂C(CH₂)₂), 52.007 (CH(X)₂C(CH₂)₂), 155.924 (OCH₂CH₂OC(=O)NH), 169.674 (CILCO,C(CIL),), 169.969 (CRCO,C(CH,),).

Example 9.

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PEG-eme-Asp-OH (12). Compound 11 (15 g. 0.375 mmol) was dissolved in DCM (150 mL) followed by the addition of TFA (75 ml.). The solution was stirred at room temperature for 2 hours and became (500 mL) added to precipitate the solid. The solid was trimusted with became to remove TFA followed by recrystallization from chilled DCM-ether. The remystallized solid was redissolved in DCM (150 mL) and washed with water (150 mL). The organic layer was separated, dried over anhydrous $MgSO_{a}$, concentrated in vacua, and precipitated with ether to give 12.4 g (83%) of product: 14C NMR 5 36.441 (CHCH₂CO₂), 50.177 (NHCH), 64.390 (OCH₂CH₂OC(=0)NH), 81.333 (CH₂CO₂C(CH₂)₂), 82.007 (CHCO₂C(CH₂)), 156.172 (OCH₂CH₂OC(=O)NH), 171.944 (CH_CO_C(CH_)_), 172.211 (CHCO_C(CH_)_).

Boe-Asp-Asp-OMe (15). EDC-HCl (2.47 g, 12.86 mmol) was added to a mixture of BocNH-capartic acid (13, 1 g. 4.29 mmol), aspartic acid dimethyl exter-HCl (14, 1.86 g. 9.43 mmol), and DMAP (2.47 g, 12.86 mmol) in anhydrous DCM (30 mL) and DMF (2 mL) at 0 °C. The mixture was allowed to warm up to mom temperature overnight. The mixture was washed with LN HCl three times and the organic layer was dried over aningdrous MgSO, followed by removal of the solvent in vacuo to give the product (2.0 g. 90%): ¹H NMR & 1.45 (s, 9H), 2.62-3.02 (m, 6H, 3 × CH), 3.70 (s, 6H, 2 × OCH), 3.74 (s, 3H, OCH,), 3.75 (s, 3H, OCH₂), 4.50 (bs, 1H, CH), 4.85 (m, 2H, 2 × CH), 6.05 (d, J = 6.95 Hz, 1H, NH), 6.98 (d, J = 8.05 Hz, 1H, NH), 7.57 (d, J = 7.69 Hz, 1H, NH).

 $\Delta sp\text{-}Asp\text{-}OMe$ (16). Compound 15 (2.0 g, 3.85 ramol) was dissolved in DCM (30 mL) and TFA (15 mL) and the solution was stirred for 2 h at room temperature. The solvent was removed in versuo and the residue was recrystallized twice with DCM-ether to give the product (1,74 g, 87%) as a white solid: "C NMR & 35.52, 48.76, 50.12, 51.90, 51.96, 52.65, 114.59, 118.49, 168.43, 170..02, 170.92, 171.17, 171.40, 171.48.

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Example 12.

PEG-cmc-Asp-Asp-Oble (17). DMAP (4.5 g, 36.86 mmol) was added to a solution of 9 (now. 40,000, 74 g, 1.34 mmol) and 16 (9.83 g, 18.43 mmol) in 700mL of anhydrous chloroform. The reaction mixture was refluxed for 24 hours under nitrogen. The reaction was cooled to room temperature and concentrated to ¼ volume. Crude product was precipinated with 2.5 L of ether, filtered and retry-stallized from 5.5 L of PA (65°C). The product was filtered and washed twice with fresh PA, twice with fresh ether, and dried overnight at 40 °C to yield 59.0g (84%) of 17: "C NMR 6 35.344, 36.931, 48.082, 48.205, 50.835, 51.509, 52.239, 61.045, 63.953, 68.854-72.056, 155.538, 170.102, 170.359, 170.453, 170.734.

Example 13.

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PEG-cure-Asp-Asp-OII (18). Compound 17 (51 g. 1.26 mmnl) and LiOII-II-JO (0.8 g. 18.9 mmnl) were dissolved in 300 ml. of water and the solution stirred overnight at room temperature. The pH of the solution was adjusted to 2.5 by the addition of 1N HCl. The solution was extracted with DCM (3 × 600 ml.), the organic layers combined, dried over unhydrous MgSO₄ and concentrated in vacuo. The residue was recrystallized from DCM-cther to give the product which was collected by filtration and dried at 40 °C overnight to yield 38 g (54%) of the octa-acid: "C NMR (D₂O) § 38.384, 39.704, 51.951, 54.465, 62.934, 67.105, 71.445-74.381 (PEO), 159.772, 173.831, 174.940, 176.359, 176.696. Example 14.

Mel-OMe (20). Melphalan (19, 1.00 g, 3.25mmol) was suspended in 2,2 dimethoxy-propone (65.59 ml., 533.49 mmol). To the suspension was added aqueous HCl (36 %, 3.28 ml.) and absolute methanol (4 ml.). The mixture was warried to mild reflux with vigorous stirring until solution started to turn slightly brown, followed by stirring at room temperature for 16 hours. The reaction mixture was comemtated fit viscuo and the crude product precipitated from the residue with other. The solid was filtered, washed with either, and purified by affice gel column chromatography (CHCl.; McOH = 9:1, Nh) to yield the desired product (0.47g, 45%): "CNMR & 39.751, 40.340, 51.912, 59.435, 55.803, 112.124, 126.076, 130.620, 145.033, 175.754.

30 Example 15.

Boc-TML-18-Mct-CMe (22). EDC (0.52 g. 2.70 mmol) and DMAP (0.988 g. 8.10 mmol) were added to a mixture of 21 (0.531 g. 1.35 mmol) and 20 (0.863 g. 2.70 mmol) in

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enhydrous DCM (15 ml.) and anhydrous DMF (5 ml.) at 0 °C in an ice bath. The reaction mixture was stirred at room temperature overslight under nitrogen then concentrated in water. The residue was redissolved in DCM (75 ml.) and washed three times with 25 ml. 1N HCl. The organic layer was dried over enhydrous tragnesium sulfare, concentrated, and purified by after gel column obromatography (ethyd sociate) chexane = 7.3, v/v) to yield the desired product (0.757 g, 80.8 %): ¹⁰C NMR 8 20.120, 23.206, 28.294, 31.768, 35.427, 35.947, 36.669, 39.505, 40.311, 49.324, 51.959, 53.234, 53.453, 79.467, 112.095, 123.374, 125.169, 130.439, 132.856, 133.427, 136.666, 138.697, 145.091, 149.841, 156.081, 170.886, 172.298.

10 Example 16.

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TML16-Mel-OMe TFA Solt (23). Compound 22 (0.757 g, 1.09 mmol) was stirred in DCM (SmL) and TFA (2.5 mL) at room temperature for 2 hours. The reaction solution was concentrated, redissolved in minimal DCM, and precipitated with other. The product was collected by filtration to yield the desired product (0.222g, 35.9 %): "C NMR (CDCI₃ + CD₂OD) 5 20.026, 25.146, 31.736, 31.592, 35.271, 36.219, 39.163, 40.340, 49.006, 52.219, 53.396, 112.073, 123.260, 124.756, 130.377, 133.026, 133.180, 136.815,

138.595, 145.110, 149.283, 171.069, 171.619, 172.630.

Example 17.

PEG-cmc-TML1β-Mei-OMe (24). A mixture of PEG-cmc-Asp-Asp-OH (12, 1.6g. 0.039 lmmol), 23 (0.277g. 0.39 lmmol), EDC (0.076g, 0.39 lmmol), and DMAP (0.155g. 1.269 nmol) in arthydrous 1XCM (23 mL) and arthydrous DMF (6 mL) was stirred overnight at room temperature under nitroger. The solution was concentrated in vector and the resultar recrystallized from 130 mL IPA to yield the product (1.543g, 92.5 %). The amount of melphalan in the product measured by UV assay was 2.86% wtwn: "C NMR & 19.642, 24.788, 31.175, 34,350, 35.975, 38.817, 39.905, 48.558, 51.553, 52.503, 60.897, 62.331, 65.145-72.878 (PEG), 111.394, 122.761, 124.425, 129.698, 132.105, 132.878, 135.804, 137.737, 144.316, 149.065, 160.432, 170.608, 171.598.

Boc-TML1β-ArnC (25). A solution of Ara-C (1, 9.58 g, 40.66 mmol) in anhydrous pyridine (85 mL) was added to a raixture of 21 (40 g, 10.17 mmol), H/Mff (5.49 g, 40.66 mmol), EDC (15.61 g, 81.32 mmol), and NMM (8.93 mL, 8.21 g, 81.32 mmol), end NMM (8.93 mL, 8.21 g, 81.32 mmol) and the majority of the following pyridine (200 mL). The reaction mixture was attred for 48 hours at 40 °C

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under nitrogen, followed by concentration in vacuo. The residue was redissolved in DCM (300 mL), washed three times with water (100 mL) and twice with 0.1N RCl (100 mL). The organic layer was dried over magnesium sulfate, concentrated, and purified by allies gel column chromatography (CHCl, --MEOH = 9:1, viv) to yield the desired product (3.26 §, 52 %): "IC NMR 6 20.315, 25.560, 28.522, 31.660, 35.520, 36.200, 39.221, 50.239, 61.719, 75.171, 76.695, 79.635, 85.341, 88.052, 96.435, 122.894, 132.519, 133.190, 136.186, 138.007, 146.222, 149.109, 155.906, 162.191, 171.733.

TMLIB-AreC TFA sait (26). Compound 25 (3 g. 4.85 tumol) was dissolved in DCM (15 ml.) followed by addition of TFA (7.5 ml.) at 0 °C. Resource mixture was stored at 0 °C for 1.2 hours and concentrated in source in a cool water bath. Residue was precipitated with DCM-other to yield the desire product (2.37 g. 77 %): °C NMR (CDCs, 1 CD,OD) 5 20.0, 25.3, 31.5, 31.7, 35.0, 38.9, 50.2, 60.9, 75.1, 75.8, 85.7, 88.1, 94.9, 109.7, 113.5, 117.3, 121.1, 122.5, 132.6, 136.4, 138.4, 148.7, 149.5, 150.1, 159.2, 159.6, 160.1, 160.6, 161.1, 170.6, 172.7

Example 20.

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PEG-cmc-Asp-Asp-TML1f-AraC, octamer (27). Compounds 26 and 18 were subjected to the same condition as in Example 18 to prepare 27.

Prepare 21

20 In vitro and in vivo data for compounds fis and 6b.

In this Example, in vivo end in vitro data are presented and compared to transdiffed Ara-C.

In Vivo

Athyrnic nucle mice were implanted subcutaneous with a 4-5 ram? tissue fragment of LX-1 collected from donor unice. The tumor trocar site was observed twice weekly and measured once pulpoble. The tumor volume for each mouse veas determined by measuring two dimensions with collipers and calculated using the formula: cumur volume = (length x width)/2. When tumors reached the average volume of 90 cm², the mice were divided into their experimental groups which consisted of unmodified Ara-C and PEG-Ara-C compounds. The mice were swited to evenly distribute numor size, grouped into 4 to 6 mice/group, and ear punched for permanent identification. Drugs were administered intravenously q3d x 4 (Dey 1, 4, 7 and 10) via the tail vein at an approximate rate of 0.5

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Ara-C was dissolved in DMSO and diluted to the appropriate concentration in culture media. The PEG-Ara-C compounds were dissolved in water and diluted in the appropriate concentrations in culture media.

The assays were performed in duplicate in 96-well microtiter cell culture plates. Two fold seriel dilution of the compounds were done in the microtiter plates. Cells were demelted by incubating with 0.1% Trypsin/Versene at 37°. Trypsin was inactivated by adding the appropriate media for each cell line containing 10% FBS. To each well of the microtiter plates, 10,000 cells were added. After three days, cell growth was measured by addition of a metabolic indicator dye, Alamat Rhie, according to the manufacturer's protocol. The ICto value for the test compounds, and reference compound are provided above in the Table.

While there have been described what are presently believed to be the preferred embediments of the invention, those skilled in the art will realize that changes and modifications may be made without departing from the spirit of the invention. It is mended to claim all such changes and modifications as fall within the true scope of the invention.

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WHAT IS CLAIMED IS:

1. A compound comprising the formula:

wherein:

$$R_1$$
 is a polymeric residue;

 Y_1 is $O_1 \cap O_2$ is $O_2 \cap O_3$.

 $R_1 \cap O_4 \cap O_4$
 $R_1 \cap O_4 \cap O_5$
 $R_1 \cap O_4 \cap O_5$
 $R_2 \cap O_4$
 $R_3 \cap O_5 \cap O_5$
 $R_4 \cap O_5 \cap O_5$
 $R_5 \cap O_6$
 $R_6 \cap O_7$
 $R_6 \cap O_7$

- (a) is zero or one;
- (m) is zero or a positive integer;
- (n) and (p) are independently 0 or a positive integer;
- $Y_{2,\gamma}$ are independently O, S or NR_{40}

R₃₋₁₉ are independently selected from the group consisting of hydrogen,

 C_{14} alkyls, C_{34} branched alkyls, C_{34} cycloalkyls, C_{14} substituted alkyls, C_{14} substituted cycloalkyls, aryls, substituted aryls, arnikyls, C_{14} beteroalkyls, substituted C_{14} beteroalkyls, C_{14} sikoxy, phenoxy and C_{14} beteroalkoxy;

D, and D₂ are independently OH,

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or a terminal branching group;

wherein (v) and (t) are independently 0 or a positive integer up to about θ_i

L, and L, are independently selected bitimetional linkers;

 $Y_{a,1}$ are independently selected from the group consisting of C_i , S and $NR_{i,c}$: $R_{1,c,b}$ are independently selected from the group consisting of hydrogen, $C_{i,d}$ alkyls, $C_{1,d}$ branched alkyls, $C_{2,d}$ cyclosikyls, $C_{1,d}$ substituted alkyls, $C_{2,d}$ substituted cyclosikyls, aryls, substituted aryls, arelkyls, $C_{1,d}$ beteroalkyls, substituted $C_{1,d}$ beteroalkyls, $C_{1,d}$ alkoxy, phenoxy and $C_{1,d}$ beteroalkyls, $C_{1,d}$ alkoxy,

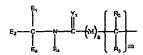
Ar is a moiety which when included in Formula (I) forms a multi-substituted arountic hydrocarbon or a multi-substituted heterocyclic group;

 B_i and B_j are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing monities or antine-containing motions.

2. The compound of claim 1, wherein R_1 further comprises a capping group A, schedule from the group consisting of hydrogen, NH₃, OH, CO₂H, C_{1-c} modeles and

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3. A compound of claim 2, commrising the formula:

4. The compound of claim 1, wherein said terminal branching group comprises the

___N___E35

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 $-\left(\begin{bmatrix} \frac{n}{2} \\ \frac{n}{2} \end{bmatrix}\right)^{\frac{n}{2}} = -c$

Enter are independently II, En or



(n) and (p) are independently 0 or a positive integer;

Y₂₀ are independently O, S or NR₁₀;

 $R_{a,b}$ are independently selected from the group consisting of hydrogen, $C_{1:a}$ alkyls, $C_{3:b}$ branched alkyls, $C_{1:a}$ cycloalkyls, $C_{1:a}$ substituted alkyls, $C_{1:a}$ substituted cycloalkyls, anyls, substituted aryle, aralkyls, $C_{3:a}$ beteroalkyls, substituted $C_{1:a}$ beteroalkyls, substituted $C_{1:a}$ beteroalkyls, substituted $C_{1:a}$

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alkyls, C_{14} alkoxy, phenoxy and C_{14} heteroalkoxy; D^*_{14} and D^*_{27} are independently OH,

wherein (v) and (t) are independently 0 or a positive integer up to about 6;

 L_1 and L_2 are independently selected bifunctional linkers;

 Y_{a+1} are independently selected from the group consisting of O, S and NR₄₊.

 $R_{\rm ti-1s}$ are independently selected from the group consisting of hydrogen,

 C_{14} alkyls, C_{24} branched alkyls, C_{23} cycloalkyls, C_{14} substituted alkyls, C_{14} substituted cycloalkyls, aryls, substituted aryls, araikyls, C_{14} heteroalkyls, substituted C_{14} heteroalkyls, C_{14} alkoxy, phenoxy and C_{14} beteroakoxy;

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Ar is a moiety which when included in Formula (1) forms a multi-substituted erametic hydrocorboo or a multi-substituted heterocyclic group;

B, and B, are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing modelies or amine-centaining modelies.

E., is

 $F_{\scriptscriptstyle \mathsf{MC-oll}}$ are independently H, E_{es} or

wherei

D", and D", are independently OH,

$$-J - \{I_J\}_{v} = \{I_J\}_{v} =$$

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- The compound of claim 3, Y₁ is O.
- 6. The compound of claim 1, wherem R_1 comprises a polyalkylene exide residue.
- The compound of claim 6, wherein R₁ comprises a polyethylene glycol residue.
- The compound of claim 3, wherem R₁ comprises a polyethylene glycol residue.
- 9. The compound of claim 6, wherem R₁ is selected from the group consisting of $C(=Y_0)$ -(CH₂), $C(H_2)$ - $C(H_2)$ -C(
- R_{13} , R_{44} and R_{25} are independently selected from among H, C_{14} alkyls, $C_{14,2}$ branched alkyls, C_{14} cycloalkyls, C_{14} substituted alkyls, C_{14} substituted C_{14} betervalkyls, anyls, substituted aryls, aralkyls, C_{14} betervalkyls, substituted C_{14} betervalkyls, C_{14} alkoxy, phenoxy and C_{14} betervalkyls;
 - e and fare independently zero, one or two; and A is a capping group.
- 10. The compound of claim 9, wherein K, comprises 40-4(CH₂CH₂C)₂ and x is a positive integer so that the weight average molecular weight is at least about 20,000.

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- 11. The compound of claim 3, wherein R_1 has a weight average molecular weight of from about 20,000 to about 100,000.
- 12. The compound of claim 3, wherein R, has a weight average molecular weight of from about 25,000 to about 60,000.
- 13. A compound of claim 3, comprising the formula

14. The compound of claim 13, wherein D, is

$$-3 - \left\{ L_{7} \right\} \left\{ L_{2} \right\} \left\{ \begin{array}{c} X_{13} & R_{16} & Y_{6} \\ \vdots & \vdots & \vdots \\ R_{14} & R_{16} \end{array} \right\} \left\{ \begin{array}{c} (IV) \\ R_{11} & R_{16} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10} \\ \vdots & X_{10} \end{array} \right\} \left\{ \begin{array}{c} X_{10} & X_{10$$

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15. The compound of claim 13, wherein D_1 is

- The compound of claim 1, wherein I₁ is (CH₂CH₂O)₂.
- 17. The compound of claim 1, wherein L₁ is selected from the group consisting of -CH₂-, · CH(CH₃)-, · CH₂C(O)NHCH(CH₃)-, · (CH₂)-, · CH₂C(O)NHCH₃-, · (CH₂)-NH-, · (CH₂
- 18. A compound of claim 1, selected from the group consisting of:

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wherein $R_{\rm t}$ is a PEG residue and D is selected from the group consisting of:

where B is a residue of an amine or a hydroxyl- containing drug.

- 19. A compound of claim 18, wherein B is a residue of a member of the group consisting of: damorubicin, doxorchicin; p-aminoenline mustard, melphalan, Ara-C (cytusine arabinoside), leucine-Ara-C, and gemeinatine
- 20. A method of treatment, comprising administering to a maximal in need of such trentment an effective amount of a compound of claim 1, wherein D_1 is a residue of a biologically active moiety.
- A method of treatment, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 18.

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22. The compound of claim I, wherein Ar comprises the formula:

wherein R_{11} and $R_{18,13}$ are individually selected from the group consisting of hydrogen, C_{14} alkyls, $C_{3,1}$ branched alkyls, $C_{3,4}$ cyclosikyls, $C_{1,3}$ substituted alkyls, $C_{3,4}$ substituted cyclosikyls, aryls, substituted tryls, aralkyls, $C_{1,4}$ beteroolkyls, substituted C_{14} beteroolkyls, $C_{1,4}$ alkory, phenoxy and C_{14} heterookoxy.

- 23. The compound of claim 22, wherein $R_{\rm H}$ and $R_{\rm then}$ are each H or CH $_{\rm 2}$
- A method of preparing a polymer conjugate, comprising: reacting a compound of the formula (VIII):

wherein

(v) and (i) are independently 0 or a positive integer up to about 6;

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 $L_{\rm t}$ and $L_{\rm p}$ are independently selected bifunctional linkers;

 Y_{44} are independently selected from the group consisting of O, S and NR $_{15}$:

 $R_{\rm titz}$ are independently selected from the group consisting of hydrogen,

 C_{14} oikyls, C_{12} branched elkyls, C_{34} eyclonikyls, C_{14} substituted nikyls, C_{14} substituted cyclonikyls, aryls, substituted aryls, atalkyls, C_{14} heterosticyls, substituted C_{14} heterostikyls, C_{14} alkoxy, phenoxy and C_{14} beterostikyls, C_{14} nikoxy, phenoxy and C_{14} beterostikyls, C_{15} nikoxy, phenoxy and C_{14} beterostikyls, C_{15} nikoxy, phenoxy and C_{15} nikoxy.

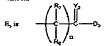
Ar is a money which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted between-plus group; and

 $\mathbf{B}^*_{\ t}$ is a residue of a hydroxyl- or an amine-containing moiety;

with a compound of the formula (IX):

$$R_1 = \begin{pmatrix} R_1 \\ C \\ R_2 \end{pmatrix} \begin{pmatrix} M \\ M \end{pmatrix}_{m} \begin{pmatrix} Y \\ C \\ M \end{pmatrix}_{m} \begin{pmatrix} C \\ C \\ C \end{pmatrix} \begin{pmatrix} C \\ C \\ C \end{pmatrix}$$

Wherei



E_{t.4} are independently H, E_t o



 D_a and D_a are independently OH, a leaving group which is cupable of reacting with an unprotected amine or hydroxyl or a terminal branching group;

R₂ is a polymeric residue;

Y, is O, S or NR.

M is O, S or NR.;

(a) is zero or one;

(m) is 0 or a positive integer;

(n) and (p) are independently 0 or a positive integer;

 $Y_{a,a}$ are independently O, S or NR $_{ab}$; and

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 $R_{p,p}$ are independently selected from the group consisting of hydrogen, $C_{1,q}$ alkyls, $C_{1,q}$ branched alkyls, $C_{1,q}$ substituted alkyls, $C_{1,q}$ substituted cycleolkyls, aryls, substituted aryls, aralkyls, $C_{1,q}$ heteroalkyls, aryls, substituted aryls, aralkyls, $C_{1,q}$ heteroalkyls, aryls, substituted $C_{p,q}$ heteroalkyls, $C_{1,q}$ alkway, phenoxy and $C_{1,q}$ betteroalkoxy; under conditions sufficient to cause a polymeric conjugate to be formed.

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Fig. 1

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Fin. 2

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Fig. 3

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Fig. 4

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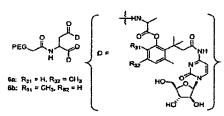
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(S4) TIBE: TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTABRING THE SAME



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TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTAINING THE SAME

PECHNICAL FUELD

The present invention relates to new types of terminally-activated polymeric materials which are useful in forming long-acting conjugates of biosective materials. In particular, the invention relates to polymeric-based conjugates having increased therepeutic psyloads and methods of preparing the same.

BACKGROUND OF THE INVENTION

Over the years, several methods of administering biologically-effective materials to maximals have been proposed. Many medicinal agents are available as water-coluble salts and can be included in phartmoentical formulations relatively easily. Problems arise when the desired medicinal egent is either insoluble in aqueous fluids or is rapidly degraded in vivo. Alkaloids are often especially difficult to solubilize.

One way to solubilize medicinal agents is to include them as part of a soluble prodrug. Prodrugs include chemical derivatives of a biologically-active parent compound which, upon administration, eventually liberate the parent compound in vivo. Prodrugs allow the artisan to modify the mast und/or duration of action of an agent in vivo and can modify the transportation, distribution or solubility of a drug in the body. Furthermore, prodrug formulations often reduce the toxicity and/or otherwise overcome difficulties, encountered when administering pharmaceutical preparations. Typical examples of prodrugs include organic phosphares or essens of alcohols or thinatochols. See Reminguols Pharmaceutical Sciences, 16th Ed., A. Ovol, Ed. (1980), the disclosure of which is incorporated by reference berein.

Produgs are often biologically mert or substantially inactive forms of the parent or active compound. The rate of release of the active drug, i.e. the rate of hydrolysis, is

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urfluenced by several factors but especially by the type of bond joining the parent drug to the modifier. Care must be taken to avoid preparing prodrugs which are eliminated through the kidney or reticular endothelial system, etc. before a sufficient emount of hydrolysis of the parent compound occurs.

Incorporating a polymer as part of a prodrug system has been suggested to increase the circulating life of a drug. However, it has been determined that when only one or two polymers of less than about 10,000 daltons each are conjugated to cartain biologically active authoraces such as alkaloid compounds, the resulting conjugates are rapidly eliminated in vivro, especially if a somewhat hydrolysis-resistant finkinge is used. In fact, such conjugates are so implify cleared from the body that even if a hydrolysis-prome extent linkage is used, not enough of the parent molecule is regenerated in NYEQ to be the expectation.

Camptotherin and reinted biologically active analogs are often poorly water soluble and are examples of substances which would benefit from PEG prodrug technology. A brief overview of some previous work in the field is presented below.

Ohya, et al., J. <u>Bionetive and Commutible Polymers</u> Vol. 10 Jan., 1995, 51-66, directose doxonabicin-PFG conjugates which are prepared by linking the two substituents via various linkages including esters. The molecular weight of the PEG used, however, is only about 5,000 at most. Thus, the <u>in vivo</u> benefits are not fully realized because the conjugates are substantially excreted grior to sufficient linkage hydrolysis.

U.S. Patent No. 4,943,579 discloses certain simple 20(S)-camptothecin amino acid esters in their salt forms as water soluble prodrugs. The reference does not, however, disclose using an amino acid as part of a linkage which would attach the silicilide to a relatively high molecular weight polymer in order to form a prodrug. As evidenced by the data provided in Toble 2 of the '579 patent, hydrobysis is rapid. Consequently, at physiologic pH, the insoluble base is rapidly generated after injection, binds to proteine and is quickly eliminated from the body before a therapeutic effect can be ochieved. A related effort was directed to developing a water-soluble camptothecin sodium salt. Unfortunately, the water-soluble sodium salt of camptothecin remained too toxic for elimical application (Gottlieb et al., 1970 Cameer Chemothers, Rep. 54, 461; Moertel et al., 1972 Did, 56, 95; Gottlieb et al., 1972 Did, 56, 103).

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Commonly-essigned PCT publication WO96/23794 describes bis-conjugates in which one equivalent of the hydroxyl-containing drug is attached to each terminal of the polymer. In spite of this advance, techniques which would further increase the payload of the polymer have been sought.

Thus, there continues to be a need to provide additional technologies for forming prodrugs of therapeutic moieties such as camptothecia and related analogs. The present invention addresses this need.

SUMMARY OF THE INVENTION

In one aspect of the invention, compounds of Formula (I) are provided:

$$R_1 = \begin{pmatrix} R_2 \\ C \\ R_3 \end{pmatrix} \begin{pmatrix} M \\ A \end{pmatrix}_a \begin{pmatrix} M \\ C \\ M \end{pmatrix}_b \begin{pmatrix} E_1 \\ E_2 \end{pmatrix} = E_2$$

wherein:

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R, is a polymeric residue;

Y, is O, S or NR.;

M is O, S or NR,

(m) is zero or a positive integer, preferably 1 or 2;

(a) is zero or one;

 $B_{p,\epsilon}$ are independently $H_{\epsilon}H_{\epsilon}$ or

(n) and (p) are independently 0 or a positive integer;

 $Y_{2,p}$ are independently O,S or $NR_{10} \hat{\cdot}$

 $R_{\rm per}$ are independently selected from the group consisting of hydrogen, C_{16} alkyls, $C_{i,ci}$ branched alkyls, $C_{i,d}$ cycloalkyls, $C_{i,d}$ substituted olkyls, $C_{i,d}$ substituted cycloality is, aryls, substituted aryls, eralityls, $C_{\rm tot}$ between lkyls, substituted $C_{\rm tot}$ between

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alkyla, $C_{i,\epsilon}$ alkoxy, phenoxy and $C_{i,\epsilon}$ beterooffcoxy;

D, and D, are independently OII,

or additional branching groups described below.

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Within formulae (IV) and (V), (v) and (t) are independently 0 or a positive integer up to about 6 and preferably about 1:

2 is NR₁₂ or

 $L_{\rm t}$ and $L_{\rm t}$ are independently selected bifunctional linkers;

 $Y_{*,*}$ are independently selected from the group consisting of O. S and NR₁₅; $R_{11,17}$ are independently selected from the group consisting of hydrogen,

 C_{14} alkyla, C_{54} branched alkyla, C_{74} cycloalityls, C_{14} substituted alkyla, C_{54} substituted

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cyclosikyis, aryis, substituted aryis, arzikyis, C_{14} beteroalkyis, substituted C_{14} beteroalkyis, C_{14} alkozy, phenoxy and C_{14} beteroalkoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

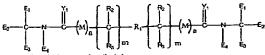
B, and B, are independently selected from the group consisting of leaving groups.

OH, residues of bydroxyl- or anine-containing moieties.

In one particularly preferred aspect of the invention, the polymeric residue is also substituted on the distal portion with a moirty of formula (II) below:

 $\mathbb{E}_{2} = \begin{bmatrix} \mathbb{E}_{1} & \mathbb{I} \\ \mathbb{E}_{3} & \mathbb{E}_{4} \end{bmatrix}^{1} - \left(\mathbb{M} \right)_{0} = \begin{bmatrix} \mathbb{R}_{2} \\ \mathbb{R}_{3} \end{bmatrix}_{m}.$

where all variables are as previously defined. Rifunctional compounds are thus formed when the polymeric residue (R₁) includes both an elpha and an omega terminal linking group so that two, four or more equivalents of a biologically active agent, drug or protein, designated herein as B₁ or D₂ can be delivered. An example of such a diffractional polymer transport form is illustrated below as formula (III):



wherein all variables are as described above.

For purposes of the present invention, the term "residue" shall be understood to mean that portion of a biologically active compound which remains after the biologically active compound has undergene a substitution reaction in which the prodrug carrier portion has been attached.

For purposes of the present invention, the term "alkyl" shall be understood to include streight, branched, substituted, e.g. halo-, alkmay-, and nitro-, C₁₋₁₁ alkyla, C₂₋₄ cyclosikyls or substituted cyclosikyls, etc.

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For purposes of the present invention, the term "substituted" shall be understood to include adding or replacing one or more atoms contained within a functional group or compound with one or more different come.

For purposes of the present invention, substituted allyla include carboxyallyls, eminonikyls, dialkylarmnos, hydroxyalkyls and mercaptoalkyla; substituted cyclonikyla include moieties such as 4-chlorocyclohoxyl; myla include moieties such as napthyl; autstituted myla include moieties such as 3-hromophenyl; aralkyla melude moieties such as tolmyl; heterocikyla include moieties such as 3-hromophene; substituted heterosikyla include moieties such as 3-hromophene; alkoxy includes moieties such as methoxy; end phanoxy includes moieties such as 3-hromophene; alkoxy includes moieties such as methoxy; end phanoxy includes moieties such as 3-hromophenexy. Halo-shall be understood to include Stuoro, chloro, todo and bromo.

The term "sufficient announts" for purposes of the present invention that mean an amount which achieves a therapeutic effect as such effect is understood by those of ordinary skill in the ert.

One of the chief advantages of the correponds of the present invention is that the profruge have a higher payload per unit of polymer than previous techniques. It is generally preferred that the polymeric first releases the trimethyl lock (TML) based prodrug intermediate by hydrolysis and then the resultant intermediate or "aecond prodrug" moiety undergoes lactionization to regenerate, for example, a moiety which is either a biologically active compound or a companion comprising a further prodrug. The high payload polymeric conjugates of the present invention are thus unique delivery systems which can contain up to four or a greater number of molecules of a drug.

Methods of making and using the compounds and conjugates described herain are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1- 5 schematically illustrate methods of forming compounds of the protent invention which are described in the Examples.

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DETAILED DESCRIPTION OF THE INVENTION

A. FORMULA (I)

In one preferred embodiment of the invention, there are provided compounds of

the formula

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 $R_1 + C + (M)_a - C$

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R, is a polymeric residue;

Y, is O. S or NR.;

M is O, S or NRs;

(a) is zero or one;

(m) is zero or a positive integer;

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 $-\left(\begin{bmatrix} \tilde{F}_7 \\ \tilde{F}_{1} \\ \tilde{F}_{0} \end{bmatrix} \right)_0^{\tilde{F}_{1}} = O_1$

 E_{i+} are independently H_i , E_i or

(a) and (p) are independently 0 or a positive integer,

 $Y_{a,b}$ are independently O, S or NR $_{10}$

 $R_{2,10}$ are independently selected from the group consisting of hydrogen,

 C_{14} alkyls, $C_{2,13}$ brouched alkyls, C_{28} cyclosikyls, C_{14} substituted alkyls, C_{24} substituted cyclosikyls, aryls, substituted axyls, aralkyls, C_{14} beteroalkyls, substituted C_{14} beteroalkyls, C_{14} alkoxy, phenoxy and C_{14} beteroalkyls, C_{15} alkoxy, phenoxy and C_{14} beteroalkyls, C_{15} alkoxy, phenoxy and C_{14} beteroalkyls, C_{15} and C_{15} alkoxy, phenoxy and C_{14} beteroalkyls, C_{15} and C_{15} and C_{15} and C_{15} are alkoxy, phenoxy and C_{14} beteroalkyls, C_{15} and C_{15} and C_{15} and C_{15} are alkoxy, phenoxy and C_{14} beteroalkyls, C_{15} and C_{15} are alkoxy, phenoxy and C_{14} beteroalkyls, C_{15} and C_{15} are alkoxy.

D, and D, are independently OH.

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v) and (f) are independently 0 or a positive integer up to about 6 and preferably about f

 \mathbf{L}_1 and \mathbf{L}_2 are independently selected bifunctional linkers;

Y_{a,5} are independently selected from the group consisting of O, S and NR₁₅, R₁₋₁₃ are independently selected from the group consisting of hydrogen.
C₁₋₄ alicyls, C₁₋₃ branched alkyls, C₁₋₆ cyclonikyls, C₁₋₄ substituted alkyls, C₂₋₆ substituted cyclonikyls, aryls, substituted aryls, aralkyls, C₁₋₆ betteroalkyls, substituted C₁₋₆ betteroalkyls, C₁₋₆ silkoxy, phenoxy and C₁₋₆ betteroalkoxy;

Ar is a moiety which when included in Formula (I) forms a mulli-substituted aromatic hydrocarbon or a multi-substituted haterocyclic group; and

 \boldsymbol{B}_1 and \boldsymbol{B}_2 are preferably independently selected from among leaving groups, OH,

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residues of hydroxyl-containing moieties or residues of amine-containing moieties.

In another preferred embodiment, D_1 and D_2 are independently selected terminal branching groups of formula (VI) E_{2n}

(VI)

wherein:

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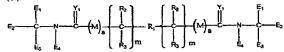
 $E_{i_3.m}$ are selected from the same group which defines $E_{i,a}$ above, except that within the definition, D_i and D_i are changed to D', and D', which are defined below. Within this embodiment, D'_1 and D'_2 can be independently OH, a moiety of formula (IV) or (V), or

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where $E_{c,m}$ are selected from the same group which defines $E_{p,o}$ except that within the definition D_1 and D_2 , are changed to D^n , and D^n , and D^n , and D^n , independently OH. formula (IV) or formula (IV). As can be apprecisted from the above, when the terminal branching is taken to its fullest extent with a bifunctional polymer R_1 , up to sixteen (16) equivalents of drug can be loaded onto the polymeric platform.

In those aspects of this embodiment where his-substituted polymeric residues are desired, some preferred polymeric transport systems of the invention are shown below as formula

ann:



wherein all variables are as previously described.

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The multi-looking polymer transport system of the present invention is based in large part on the polymerio residue designated herein as R_r. Optionally, R_t includes a cusping group A. The polymer capping group A includes, for example, modelles such as hydrogen, CO₂H, C_{1,4} alley! moieties, and compounds of formula (II) shown below, which forms a bis-system:

(II)

$$E_2 = \begin{bmatrix} E_1 & & & \\ & & \\ & & \end{bmatrix}_0^1 - \begin{pmatrix} M \end{pmatrix}_a \begin{bmatrix} R_2 \\ & \\ R_3 \end{bmatrix}_m$$

wherein all variables are as previously described. It will be understood and appreciated that the multiple terminal branching described above applies equally in the bis-systems as well.

With regard to the other veriables which comprise the formulae of the present invertion, the following are preferred:

Y_{1.1} are each exygen;

 $R_{2.10}$ and R_{42} are each preferably hydrogen or lower alkyl, e.g. $C_{\rm tai}$

 R_{11}, R_{13} and R_{14} are preferably -CH₃;

(m) is 1 or 2;

(n) and (p) are each either zero or an integer from 1-4;

(r) is zero or 1;

(i) is 1;

L, is -(CH2CH,O),-; and

 $\label{eq:local_local_local} 1_2 \text{ is one of -CH}_{27} - \text{CH}(\text{CH}_3)_2 - \text{CH}_2)_3 - \text{CH}_3)_2 \text{NH-}_3 - \text{CH}_4 \text{C}(\text{O}) \text{NHCH}(\text{CH}_4)_2 - \text{CH}_3)_2 \text{NH-}_4 - \text{CH}_4 \text{C}(\text{O}) \text{CH}_3)_2 \text{NH-}_4 \text{CH}_4 \text{CH}_3)_2 - \text{CH}_4 \text{C}(\text{O}) \text{NHCH}(\text{CH}_3)_3 - \text{CH}_4 \text{C}(\text{O}) \text{NHCH}(\text{CH}_3)_3 \text{NH-}_4 \text{CH}_4)_2 - \text{CH}_4 \text{C}(\text{O}) \text{NHCH}(\text{CH}_3)_3 \text{NH-}_4 \text{CH}_4)_2 - \text{CH}_4 \text{C}(\text{O}) \text{NHCH}(\text{CH}_3)_3 \text{NH-}_4 \text{CH}_4)_2 - \text{CH}_4 \text{C}(\text{O}) \text{NHCH}(\text{CH}_4)_3 \text{NH-}_4 \text{CH}_4)_3 + \text{C}(\text{C}(\text{O}) \text{C}(\text{C}(\text{O}) \text{CH}_4)_3 + \text{C}(\text{C}(\text{O}) \text{C}(\text{C}(\text{C}(\text{O}) \text{C}(\text{C}(\text{O}) \text{C}(\text{C}(\text{C}(\text{O}) \text{C}(\text{C}(\text{O}) \text{C}(\text{C}(\text{C}(\text{O}) \text{C}(\text{C$

B. DESCRIPTION OF THE A. MOTELY

Referring to Formula (I), it can be seen that the Ar is a moiety, which when included in Formula (I), forms a multi-substituted aromatic hydrocarbon or a multi-substituted between the group. A key feature is that the Ar moiety in aromatic in nature. Generally, to be aromatic, the melectrons must be shared within a "cloud" both above and below the plane of a cyclic molecule. Furthermore, the number of a electrons must satisfy

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the Hückel rule (4n+2). Those of ordinary skill will realize that a myriad of moietles will satisfy the aromatio requirement of the moiety and thus are suitable for use herein. One particularly preferred aromatic group is:

wherein R_{1820} are selected from the same group which defines R_{11} . Alternative aromatic groups include:

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wherem and Z_i and Z_p are independently CR_{2i} or NR_{2i} ; and Z_i is O_s S or NR_{2i} where R_{skri} at o selected from the same group as that which defines $R_{\rm cl}$ or a cyano, mito, carboxyl, acyl, substituted acyl or earliercyalkyl. Isomers of the five and six-membered rings are also contemplated as well as benzo- and dibenzo- systems and their related congeners are also contemplated. It will also be appreciated by the artisan of ordinary skill that the aromatic rings can optionally be substituted with hetero-cloms such as O, S. NR₂₁, etc. so long as Hückel's rule is obeyed. Furthermore, the aromatic or heteroryelic structures may optionally be substituted with halogen(s) and/or side chains as those terms are community understood in the art. However, all structures suitable for As moieties of the present invention are capable of allowing the B_i or B_j containing motivies and the $(R_{\rm H})$ motivity to be in an ortho arrangement with the same plane.

DRUG GENERATION VIA HYDROLYSIS OF THE PRODRUG

The prodrug coropounds of the present invention are designed so that the $t_{\rm kc}$ of hydrolysis is $< t_{1/2}$ elimination in plasme.

The linkages included in the compounds have hydrolysis rates in the plassms of the mammal being treated which is short enough to allow sufficient amounts of the parent compounds, i.e. the amino- or hydroxyl-containing bioactive compound, to be released prior to elimination. Some preferred compounds of the present invention have a t_{th} for hydrolysis in plasma ranging from about 5 minutes to about 12 hours. Preferably, the compositions have a plasma to hydrolysis runging from about 0.5 to about 8 hours and most preferably from about 1 to about 6 hours.

SUBSTANTIALLY NON-ANTIGENIC POLYMERS

As stated above, \mathbf{R}_1 is a water soluble polymeric residue which is prefembly substantially non-antigenic such as a polyalkylene oxide (PAO) or polyethylene glycol (PEG). In preferred aspects of the invention, R, further includes the previously mentioned capping group, designated herein as A, which allows a bifunctional or bis-polymer system

As an example, the PEG residue portion of the inventive compositions can be selected from the following non-limiting list: -C(=Y,)-(CH,)-O-(CH,CH,O),-A,

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-C(=Y₄)- Y, -(CH₃)_P-O-(CH₃CH₃O)₃-A,
-C(=Y₄)-NR₂₂-(CH₃)_P-O-(CH₂CH₃O)₃-A,
-(CR₄B₂₃)₃-O-(CH₃)₃-O-(CH₂CH₃O)₃-A,
-NR₂₂-(CH₃)₃-O-(CH₃CH₃O)₃-A,
-(C(=Y₄)-(CH₃)₃-(CH₃)₃-(CH₃)₃-(CH₃)₃-Y-C(=Y₄)₃.
-(C(=Y₄)-Y₂-(CH₃)₃-O-(CH₃CH₃O)₃-(CH₃)₃-Y-C(=Y₄)₃-(C(=Y₄)-NR₂₂-(CH₃)₃-O-(CH₃CH₃O)₃-(CH₃)₃-Y-C(=Y₄)₃-(CR₄)₃R₂₁-(O-(CH₃)₃-O-(CH₃)₃-(CH₃)₃-C(=Y₄)₃-(CR₄)₃R₂₁-(O-(CH₃)₃-O-(CH₃)₃-(CH₃)₃-C(CR₃)₃-1 and
-NR₂₂-(CH₃)₃-O-(CH₃)-(CH₃)-(CH₃)₃-NR₃₂-C(-X₃)₃-1 and
-NR₂₂-(CH₃)₃-O-(CH₃)-(CH₃)-(CH₃)₃-NR₃₂-C(-X₃)₃-1 and
-NR₂₂-(CH₃)₃-O-(CH₃)-(CH₃)-(CH₃)-NR₃₂-C(-X₃)₃-1 and
-NR₂₂-(CH₃)-(CH₃)-(CH₃)-(CH₃)-(CH₃)-NR₃₂-C(-X₃)₃-1 and
-NR₂₂-(CH₃)-(CH₃)-(CH₃)-(CH₃)-(CH₃)-NR₃₂-C(-X₃)₃-1 and
-NR₂₂-(CH₃)-(CH₃)-(CH₃)-(CH₃)-(CH₃)-NR₃₂-C(-X₃)-1 and
-NR₂₂-(CH₃)-(CH₃)-(CH₃)-(CH₃)-(CH₃)-NR₃₂-C(-X₃)-1 and
-NR₂₂-(CH₃)-(CH

wherein Y_a and Y_7 are independently $O_{\nu}S$ or $NR_{12};$

 \mathbf{x} is the degree of polymerizations

 R_{2p} , R_{2q} and R_{cp} are independently selected from among H. $C_{1,q}$ alkyls, $C_{p,q}$ branched alkyla, $C_{2,q}$ cyclosikyls, aryls, substituted cryls, aralkyls, $C_{p,q}$ aktoroalkyls, aryls, substituted cryls, aralkyls, $C_{p,q}$ aktoroalkyls, substituted $C_{1,q}$ heteroalkyls, $C_{1,p}$ alkoxy, phenoxy and $C_{1,q}$ beteroalkoxy;

e and f are independently zero, one or two; and

A is a capping group.

The degree of polymerization for the polymer (a) can be from about 10 to about 2,300. This represents the number of repenting units in the polymer chain and is dependent on the molecular weight of the polymer. The (A) molecy is a capping group as defined breim, i.e. a group which is found on the terminal of the polymer and, in some asperts, can be selected from any of H, NH₂,OH, CO₂H, C_{1,4} alkyls or other PEG terminal activating groups, as such groups are understood by those of ordinary skill.

Also useful are polygropylene glycots, branched PEG derivatives such as those described in commonly-assigned U.S. Patent No. 5.643,575, "star-PEG's" and multi-armed PEG's such as those described in Shearwater Polymera, Inc. caulog "Polyethylene Glycol Derivatives 1997-1998". The disclosure of each of the foregoing is incorporated herein by reference. It will be understood that the water-soluble polymer can be functionalized for attachment to the bifunctional linkinge groups if required without undue experimentation.

to a further embodiment R, is optionally selected from among one or more of dextran, polyvinyl alcohols, carbolydrate-based polymers, hydroxypropylmethacryl-

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amide, polyalkylene oxides, and/or copolymers thereof. See also commonly-essigned U.S. Patent No. 6,153,655, the contents of which are incorporated herein by reference.

In many espects of the present invention, big-activated polyethylene glycols are preferred when di-or more substituted polymer conjugates are desired. Alternatively, polyethylene glycols (PEG's), mono activated, C₁₋₄ alkyl-terminated polyethylene oxides (PAO's) such as mono-methyl-terminated polyethylene glycols (mPEG's) are preferred when mono-substituted polymers are desired.

In order to provide the decired by drulyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids can be used as well as mono- or di-PEG amines and mono- or di-PEG duals. Suitable PAO acids can be synthesized by first converting mFEG-OH to an ethyl exer followed by suponification. See also Gethrardt, H., et al. Polymer Bulletin 18: 487 (1987) and Veroness. F.M., et al., J. Controlled Release 10; 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mFEG-OH into a r-buyl ester followed by soid cleavage. See, for example, commonly assigned U.S. Paterti No. 5,605,976. The disclosures of each of the foregoing are incorporated by reference herein.

Although PAO's and PEO's om vary substantially in average molecular weight, the polymer partion of the prodrug is at least about 20,000 weight average in most aspects of the invention. Preferably, R, has a weight average molecular weight of form about 20,000 to about 100,000 and more preferably from about 25,000 to about 60,000. The average molecular weight of the polymer selected for hollusion in the prodrug must be sufficient so as to provide sufficient circulation of the prodrug before hydrolysis of the linker.

The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polymopylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

As an alternative to PAO-based polymers, effectively non-antigonic materials such as dextran, polyvinyl eloohols, carbohydrate-based polymers, bydroxypropylmethacrylamide (HPMA), and copolymers thereof etc. and the like can be used if the same type of activation is employed as described herein for PAO's such as

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PEG. Those of ordinary skill in the art will realize that the furegoing list is merely illustrative and that all polymeric materials having the qualities described herein are contemplated. For purposes of the present invention, "effectively non-emigenic" and "substantially non-emigenic" shall be understood to include all polymeric materials understood in the art as being substantially non-toxic and not eliciting an appropriable immane response in manageals.

It will be clear from the foregoing that other polyalkylene oxide derivatives of the foregoing, such as the polypropylene glycol acids, cit., as well as other bi-functional linking groups are also contemplated.

E. PRODRUG CANDIDATES

Residuce of Hydroxyl-containing Compounds

s. Camptelbecin and Related Topoisoperase I Inhibitory

Camptotheem is a water-insoluble cytobraic alkaloid produced by Camptotheen accuminate trees indigenous to China and nothapochyse footien trees indigenous to India. Camptotheeln and related compounds and analogs are also known to be potential unticencer or antitumor agents and have been shown to exhibit these activities in vivo and in vivo. Camptotheein and related compounds are also candidates for conversion to the prodrugs of the present invention.

Campiothecin and certain related analogues share the structure:

From this care structure, several known analogs have been prepared. For example, the A ring in either or both of the 10- and 11-positions can be substituted with an OH. The A ring can also be substituted in the 9-position with a straight or branched $C_{i,\infty}$ alkyl or C_{k+1} alknow, optionally linked to the ring by a beteroatum i.e.- O or S. The B ring can be substituted in the 7-position with a straight or branched $C_{i,\infty}$ alkyl or substituted 2lkyl-, $C_{i,\infty}$ elkyl $C_{i,\infty}$ alkyl, phenyl alkyl, etc., alkyl carbamate, alkyl

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carbazides, phenyl hydrazine derivatives, quatroc, aminos flayl, araflayl, etc. Other substitutions are possible in the C, D and E rings. See, for example, U.S. Peteni Nos. 5,004,758; 4,943,579; Re 32,518, the contents of which are incorporated herein by reference. Such derivatives can be made using known synthetic techniques without undue experimentation. Preferred camprothesin derivatives for use herein include a 20-OH or mother OH matery which is capable of reserting directly with activated forms of the polymer transport systems described herein or to the linking moiety intermediates, e.g. iminodiacetic acid, etc., which are then attached to a polymer such as PEG.

Reference to comproduce in smallegs herein has been made for purposes of illustration and not limitation.

Taxanes and Paclitate! Derivatives

One class of compounds included in the prodrug compositions of the present invention is taxanes. For purposes of the present invention, the term 'taxanes' includes all compounds within the taxane furnity of terpenes. Thus, taxol (pacitaxel), 3-substituted farti-buloxy-carbonyl-smine derivatives (matches) and the like as well as other analogs which are rendily synthesized using standard organic techniques or are available from commercial sources such as Sigma Chemical of St. Louis, Missouri are within the scope of the present invention. These derivatives have been found to be effective anti-cancer agents. Numerous stallies indicate that the agents have activity against several realignancies. To date, their use has been sewerely limited by, among other things, their shurt supply, poor water solubility and a tendency to cause hypersensitivity. It is to be understood that other taxanes including the 7-aryl-carbonates and 7-carbonates disclosed in commonly assigned U.S. Patent Nos. 5.622,986 and 5,547,981 cm also be included in the prodrugs of the present invention. The contents of the foreigning U.S. patents are incorporated herein by reference. Published is a preferred taxane.

c. Additional Biologically-Active Moieties

In addition to the foregoing molecules, the prodrug formulations of the present invention can be prepared using many other compounds. For example, biologically-active compounds such as his-PEG conjugates derived from compounds such as

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triazoic-based antifungal agents such as fluormazole:

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The parent compounds selected for pruding forms need not be substantially water-intelluble, elthough the polymer-based prodrugs of the present invention are especially well suited for delivering such water-insoluble compounds. Other useful parent compounds include, for example, certain low nolecular weight biologically active proteins, enzymes and poptides, including poptide glycans, as well as other anti-tumor agents; cardiovascular agents; cardiovascular agents; cardiovascular agents, cauch as forskolin; anti-ocoplastics anch as ombretastatin, viriblastine, doxorubicite, maytansine, cac; anti-infectives such as vancomycin, crythromycin, etc.; asti-fungals such as mystatin, amphocaricin B, triazoles, papulocandins, pneumocandins, ochinocandins, polyoxins, nikkomycius, pradimicius, benanomicins, etc. sec. "Antibiotios That Inhibit Fungal Cell Wall Development" Annu. Rev Microbiot. 1994, 48:471-97, the contents of which are incorporated herein by reference; auti-anxiety agents, pastrocatestinal agents, central nervous system-activating agents, unalgecies, fertility or contraceptive agents, central nervous system-activating agents, anti-uneversica agents, cardiovascular agents, vesodilating agents, vasoconstricting agents and the like.

The foregoing is illustrative of the biologically active moieties which are suitable for the prodrugs of the present invention. It is to be understood that those biologically active materials not specifically menhanced but having suitable ester-forming groups, i.e. bydroxyl moieties, are also intended and are within the scope of the present invention. It is also to be understood that the prodrag conjugates of the present invention may also include minor amounts of compounds containing and only one equivalent of drug and polymer but also a moiety which does not effect bioactivity in vivo. For example, it has been found that in some instances, in spite of reacting discids with drug molecules having a single linkage point, the reaction conditions do not provide quantitative amounts of prodrugs with two equivalents of drug per polymer. By-products of the rescants can sometimes be formed such as acyl means if carbodilinides are used.

2. Residues of Ambre containing Compounds

In some aspects of the invertion, B, or B₂ is a residue of an umine-containing compound, a non-limiting list of such suitable compounds include residues of arganic compounds, enzyroes, proteins, polypeptides, etc. Organic compounds include, without limitation, moistics such as anthracycline compounds including daunorubicin, dexorubicin, p-uminomiline mustard, melphalan. An-C (cytoxine arabinoside) and

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related anti-metabolite compounds, e.g., genetiabine, etc. Abarnatively, B can be a residue of an armino-containing cardiovascular agent, anti-neoplastic, anti-inflective, anti-fungal such as nystatin and amphoterioin B, anti-anxiety agent, grastrointestinal agent, central nervous system-activating agent, analyssic, farility agent, contraceptive agent, anti-inflammatory agent, receival agent, anti-orecemic agent, vasodilating agent, vasodonaricting agent, etc.

In a preferred aspect of the invention, the amino-centaining compound is a biologically active compound that is suitable for medicinal or diagnostic use in the treatment of animals, e.g., manufuls, including humans, for conditions for which such treatment is desired. The foregoing list is meant to be illustrative and not limiting for the compounds which can be modified. Those of ordinary skill will realize that other such compounds can be similarly recodified without undue experimentation. It is to be understood that these biologically active contents not specifically mentioned but having suitable amino-groups are also intended and are within the scope of the present invention.

The only limitations on the types of smine-containing molecules anitable for inclusion herein is that there is available at least one (primary or secondary) amine-containing position which can react and link with a carner portion and that there is not substantial loss of bioactivity after the product system releases and regenerates the parent correpound.

It is noted that parent compounds suitable for incorporation into the prodrug compositions of the invention, may themselves be substances/compounds which are not active after hydrolytic release from the bushed composition, but which will become active after undergoing a further chemical process/teaction. For example, an anticancer drug that is delivered to the bloodstream by the double prodrug transport system, may remain inactive until entering a cancer or tumor cell, whereupon it is activated by the current or tumor cell chemistry, e.g., by an enzymatic reaction ampute to that cell.

3. Leaving Groups

In those aspects where B, or B, is a leaving group, anitable leaving groups include, without limitations, moieties such as N-hydroxybenzotriazolyl, halogen, N-hydroxyphthalizaidyl, p-nitrophenoxy, innitazolyl, N-hydroxysuccinimidyl; thinzolidinyl thione, or other good leaving groups as will be apparent to those of ordinary

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skill. The synthesis resultors used and described herein will be understood by these of ordinary skill without undue experiments ton.

For example, an acytated intermediate of compound (I) can be reacted with a reactant such as 4-nitruphenyl chloroformats, disaccinimidyl carbonate (USC), carbonyldiknidazole, thiambliding thione, etc. to provide the desired activated derivative.

The selective asylation of the phenolic or emilinic parties of the p-hydroxybenzyl alcohol or the p-anniobenzyl alcohol and the o-hydroxbenzyl alcohol or the o-anniobenzyl alcohol can be curied out with, for example, thissoliding thione activated polymers, succinimital carbonate activated polymers, succinimital carbonate activated polymers, blocked amino unid derivatives. Once in place, the "activated" form of the PEG product (or blocked product) is ready for conjugation with an amino- or hydroxyl-containing compound.

P. SYNTHESIS OF THE POLYMERIC PRODRUG TRANSPORT SYSTEM

Synthesis of representative polymer products is set forth in the Examples. Generally, however, in one preferred method of preparing the product transport systems, the polymer residue is first statched to the branching groups. Separately, the biologically active moiety or drug, e.g. Drug-Oli or Drug-Nil, (R, or B, of formula I) is attached to the TML coruponent which may also include a bifunctional spacer thereon at point of attachment to the polymer. Next, the polymeric residue containing the terminal branches is reacted with the drug-TML portion under conditions sufficient to form the final product.

Attachment of the bifunctional spacer containing the TML-Drug component to the polymer portion is preferably carried out in the presence of a coupling agent. A non-limiting list of suitable coupling agents include 1,3-ditropropylearboditmide (DIPC), any suitable distlyd carboditmides, 2-thalo-1-olleyl-pyridinium haldees, (Motayama reagents), 1-(1-dimethylaminopropyl)-3-ethyl carboditmide (EDC), propone phosphonic acid cyclic unhydride (PPACA) and phonyl dichlorophus-photos, etc. which are available, for example from commercial sources such as Sigma-Aldrich Chemical, or synthesized using known techniques.

Preferably the substituteds are reacted in an inert solvent such as methylene chloride, chloroform, DMF or mixtures thereof. The reaction also preferably is conducted

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in the presence of a base, such as dimethylammopyridine, diisopropylethylamine, pyridine, triethylamine, etc. to neutralize any acids generated end at a temperature from 0°C up to about 22°C (room temperature).

More particularly, one method of preparing a polymeric transport system includes reacting a compound of the formula (VIII):

wherein all variables are as previously defined and

B', is a residue of a hydroxyl- or an amine-containing unolety; with a compound of the (ormula (TX):

$$R_{i} = \begin{bmatrix} R_{i} \\ R_{i} \end{bmatrix} \begin{bmatrix}$$

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wherein

R_i is a polymeric residue; Y_i is O_i S or NR_i; M is O_i S or NR_i; (a) is zero or one; (m) is 0 or a positive integer, $Y_{2,1}$ are independently O, S or NR_{10} and $R_{\rm to}$ are independently selected from the group consisting of hydrogen, \mathbf{C}_{14} alkyls, \mathbf{C}_{22} branched alkyls, $C_{3,4}$ cycloalkyls, $C_{1,4}$ substituted alkyls, $C_{3,4}$ substituted cycloalkyls, aryls, substituted anyle, smallyle, C_{14} betwoodkyle, substituted C_{14} betwoodkyle, C_{14} alkowy, phenoxy and C;4 heteroalkoxy;

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E, is
$$\begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix} \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix} \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix}$$

 $E_{s,a}$ are independently $\Pi_s E_s$ or

wherein D_2 and D_4 are independently OR or a leaving group which is capable of reacting with an unprotected amine or hydroxyl or a terminal branching group;

(n) and (p) are independently 0 or a positive integer,

Y_{3.3} are independently O, S or NR_{rei} and

 R_{bab} we independently selected from the group consisting of hydrogen. $C_{1,d}$ alkyls, $C_{1,d}$ branched alkyls, $C_{1,d}$ oveloalkyls, $C_{1,d}$ substituted alkyls, $C_{2,t}$ substituted cycloalkyls, aryls, substituted aryls, aralkyls, $C_{1,d}$ heteroalkyls, $C_{1,d}$ alkoty, phenoxy and $C_{1,d}$ heteroalkyls, $C_{1,d}$ alkoty, $C_{1,d}$ alk

In further aspects of the method, \mathbf{D}_{i} and \mathbf{D}_{i} are independently selected terminal

branching groups of formula (X)

where E_{11-D} are selected from the same group which defines E_{140} , except that D_1 and D_4 are changed to D_1 and D_2 which are defined below. Within this embodiment, D_1 and D_2 and D_3 are independently OH, a motory of formula (IV) or (V), or (XI)

wherein P_{2234} are selected from the same group which defines E_{37a} except that D_3 and D_4 are changed to D^a_3 and D^a_4 , which are defined as being independently OH or a leaving group which is capable of reacting with an unprotected arrans or hydroxyl.

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Such synthetic techniques allow up to nixteen (16) equivalents of carboxytic acid or activated carboxytic acid, for example, to be attached. As shown in the preferred attractures beroin, PEO residues with terminally branched multi-acids are preferred aspects of the invention.

Regardless of the synthesis selected, some of the preferred compounds which result from the synthesis techniques described herein include:

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wherein R_i is a pulymer residue such as a PAO or PEG and D is OH, formula (IV) or (V). Preferably, D is

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where B is a residue of an amine or a hydroxyl- containing drug.

In another preferred aspect of the invention, the compounds of the present invention are of formula (VU):

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wherein all variables are as previously defined above.

G. IN VIVO DIAGNOSTICS

A further aspect of the invention provides the conjugates of the invention optionally prepared with a diagnostic tag linked to the transport enhancer described above, wherein the tag is selected for diagnostic or maging purposes. Thus, a soitable tag is prepared by linking any suitable moiety, s.g., on armin acid residue, to any art-standard cruiting isotope, radio-opaque label, magnetic resumance label, or other non-radioactive isotopic labels suitable for magnetic resonance imaging, fluorescence-type labels, labels exhibiting visible colors and/or espable of fluorescing under ultraviolet, infrared or electrochemical stimulation, to allow for imaging tumor tissue during surgical procedures, and so forth. Optionally, the diagnostic tag is incorporated into and/or linked to a conjugated therapeatic moiety, allowing for moritoring of the distribution of a therapeutic biologically series unaterial within an animal or human patient.

In a still further aspect of the invertion, the inventive tagged conjugates are readily prepared, by art-known methods, with any suitable label, including, e.g., radioisotope labels. Simply by wny of example, these include "Flodine, The state of the second second second to produce radioimmunoscintigraphic agents for selective uptake into lumor cells, in vivo. For instance, there are a number of art-known methods of linking peptide to Te-99m, including, simply by way of example, those shown by U.S. Patent Nos. 5,328,679, 5,888,474; 5,997,844; and 5,997,845, incorporated by reference

Broadly, for anatomical localization of turner tissue in a patient, the conjugate tag is administered to a patient or minual suspected of having a turner. After sufficient time to allow the labeled immunoglobulin to localize at the turner site(v), the signal generated by the label is detected, for instance, visually, by X-ray radiography, computerized transaxial tomography, MRI, by instrumental detection of a luminescent tag, by a photo scauning device such as a gamma camera, or any other method or instrument appropriate for the nature of the selected tag.

The detected signal is then converted to an image or enutomical antifor physiological determination of the tumor site. The image makes it possible to locate the tumor in who and to device an appropriate therapentic strategy. In those embodiments

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where the tagged moiety is itself a therupeutic agents, the detected aignal provides evidence of anatomical localization during treatment, providing a baseline for follow-up diagnostic and therapeutic interventions.

FI. METHODS OF TREATMENT

Another aspect of the present invention provides methods of treatment for various medical conditions in mammals. The mothods include administering to the mammal in need of such breatment, an effective amount of a product, such as a multi-loaded Ara-C-PEG conjugates, which has been prepared as described herrin. The compositions are useful for, among other things, treating neoplastic disease, reducing himser burden, preventing metastassis of neoplastics and preventing recurrences of times/neoplastic growths in mammals.

The amount of the prodrug administered will depend upon the parent molecule included finerin. Generally, the amount of prodrug used in the treatment methods is that expount which effectively achieves the desired therapeartic result in mammals. Naturally, the designs of the various prodrug compounds will vary somewhat depending upon the purent compound, rate of in vivo hydrolysis, molecular weight of the pulymar, etc. In general, however, prodrug taxanes are administered in amounts ranging from about 5 to about 500 mg/m² per day, based on the amount of the texane moiety. Camptotherein prodrugs are also administered in amounts ranging from about 5 to about 500 mg/m² per day. The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the prodrug selected based on othical experience and the treatment tridication. Actual designs will be apparent to the ortion without undue experienciation.

The prodrugs of the present invention can be methoded in one or more suitable pharmaceutical compositions for administration to manurals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, especie or the like, prepared according to methods well known in the srt. It is also contemplated that administration of such compositions may be by the oral and/or parentaral routes depending upon the needs of the artisam. A solution and/or parentaral routes may be utilized, for example, as a carrier vehicle for injection or infiltration of the composition by any ert known methods, e.g., by intravenous, intramuscular, subdermal injection and the like.

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Such administration any also be by infusion into a body space or cavity, as well as by infusiation and/or intrunsed routes. In preferred aspects of the invention, however, the prodrugs are parenterally administrated to commands in need thereof.

. EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention. The underlined and bold-fixed numbers recited in the Examples correspond to those shown in Figures 1-

General. All reactions were run under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. All PEG compounds were dried under vacuum or by azentropic distillation (tolurare) prior to use. 'El spectra were obtained with a JEOL FT NMR System JNM GSX-270 instrument using deuteriochloroform as solvent unless specified. "C NMR spectra were obtained at 67.80 MHz nn the JNM GSX-270. Chemical shifts (8) are reported in parts per million (ppm) downfield from tetremethylallane (TMS) and coupling constants (J values) are given in Lertz (Hz). All PEG conjugated compounds were dissolved (~15 mg/ml.) in sterile saline (0.9%) for injection prior to in vivo drug treatments and were given as their ara-C equivalents (absolute amount of ara-C given).

HPLC Method. Analytical HPLC's were performed using a CB reversed phase column (Beckman, ultrusphere) under isocratic conditions with an 80:20 tubxhure (v/v) of methanol-water as mobile phase. Peak elutions were monitored at 254 and using a UV detector. To detect the presence of any free PEG and also to confirm the presence of PEGYLATED product, an evaporative light scattering detector (ELSD), Model PL-EMD 950 (Polymer Laboratories), was amployed. Based on ELSD and UV analysis, all the final PEGytated products were free of native drug and were 2 95% pure by HPLC. Analysis of Ara-C Content to PEG Berlyatives. For the determination of the ara-C content in PEG derivatives, N-acctyleytidine was used as a model. The UV absorbance of N*-acctyleytidine in R₂O was determined at 257 nm for six different concentrations concentrations. the absorption coefficient, e, of N*-acctyleytidine was calculated to be 36.4 (O.D. at 257 nm for 1 mg/mL with 1.0 cm light path). PEGylated ara-C derivatives were

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dissolved in $\rm H_2O$ at an approximate concentration of 0.015 $\mu mol/mL$ (based as a MW of 40 kDa) and the UV absorbance of these compounds at 257 nm was determined. Using this value and employing the absorption coefficient, c, obtained from the above, the concentration of ara-C in the sample was determined. Dividing this value by the sample concentration provided the percentage of ara-C in the sample. Analysis of Melphalan Content in PEG Derivatives. For the determination of the metphalan content in PEG derivatives, metphalan was used as a standard. The UV absorbance of melphalan in DMF-H₂O (9:1, v/v) was determined at 264 nm for five different concentrations ranging from 0.02 µmol/ml, to 0.06 µmol/ml. From the standard plot of absorbance us. concentration, the absorption coefficient, e, of melphalan was calculated to be 54.6 (O.D. at 264 mm for 1 mg/ml. with 1.0 cm light path). PEGYLATED melphalan derivatives were dissolved in DMP-R₂O (9:1, v/v) at an approximate concentration of 0.013 $\mu mol/mL$ (based on a MW of 40 kDz) and the UV absorbance of these compounds at 264 mm was determined. Using this value and entrploying the absorption coefficient, c, obtained from the above, the concentration of inclphalan in the sample was determined. Dividing this value by the sample concentration provided the percentage of malphalan in the sample. Abbreviations. DCM (dichloromethane), DMAP (4-(dimethylamino)pyridine), EDC (1ethyl-3-(3-dimethylanimopropyl)carbudiimide), HOET (1-hydroxybenzotriazolt), IPA (2proposed), NMM (N-methylmorpholine), TFA (trifluorozoetic sold). Example 1. Computed 3a. A mixture of ara-C (1, 1.73 g, 7.12 mmol), 2a (700 mg, 1.78 mmol), HOBT (0.96 g, 7.12 mmol), and EDC-HC! (2.73 g, 14.25 mmol) in anhydrous pyridine (50 mL) was stirred at room temperature for 2 h, the temperature raised to 40 °C and the reaction continued overnight. The solvent was removed, methylene chloride (50 mL) was used to dissolve the mixture followed by washing with water (3 \times 30 mL) and then with 0.1 N HCl (2 × 30 mL). The organic layer was dried over auhydrous MgSO, and the solvent removed in vacuo to give the crude product which was purified by silica go? column chromatography (5 to 10% MeOH in DCM) to give 638.8 mg (52%) of 3a as a

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5.49, 6.07, 6.17, 6.52, 6.76, 7.31, 7.67, 8.16, 8.62; ¹²C NMR & 17.77, 20.11, 25.36, 28.32, 31.51, 31.96, 39.57, 50.18, 50.45, 61.88, 74.50, 80.15, 85.90, 88.58, 96.25, 122.51,

white solid: 1H NMR 8 1.42, 1.55, 2.17, 2.26, 2.46, 2.79, 3.84, 3.91, 4.14, 4.33, 4.53,

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132.32, 133.34, 136.73, 138.22, 146.57, 149.90, 155.65, 155.96, 162.08, 171.89, 174.06. Kremple 2.

Compound 3h. Compound I was coupled with Zh using a similar condition as in Example 1 to produce 3b in 54% yield: ¹³C NMR 8_17.23, 17.92, 18.33, 25.49, 28.32, 31.51, 31.58, 31.99, 22.46, 39.52, 40.09, 50.08, 50.22, 61.72, 74.50, 74.94, 80.11, 80.15, 85.45, 85.90, 88.01, 88.58, 96.25, 122.51, 126.77, 129.03, 129.16, 131.68, 132.82, 136.24, 136.73, 138.22, 146.05, 146.57, 149.90, 155.65, 155.96, 171.85, 171.89, 174.06. Example 3.

Compound 4a. Compound 3a (638.8 mg. 1.03 mmol) was stirred in anhydrous DCM (6 mL) and TPA (4 mL) at room temperature for 2 h. Bibyl other was added to the solution to precipirate the crude product which was filtered and washed with other to give 4s as a write solid (534.5 mg, 82%): "H NMR (DMSO-d₂) & 1.52 (s, 3H, (CH₂)CH) 1.55 (s, 3H, (CH₂)CH), 1.62 (d, 1 H, J = 8.1 Hz, (CH₂)CH), 2.22 (s, 3H, CH₂)CH, 2.77 (s, 3H, CH₂)CH), 2.97 (s, 2H, CH₂)C(CH), 3.41-4.27 (m, 5 H, an-C's II-2'-HS'), 6.09 (d, 1H, J = 5.4, sna-C's II-1'), 6.67 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.12 (d, J = 5.4, H-6), 8.05 (d, J = 8.1, H-5), 8.67 (bs, 1H, TFA); "C NMR (DMSO-d₂) & 15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 35.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.88,

20 Example 4.

171,87.

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Compound 4b. Compound 3b was subjected to the same condition as in Example 3 to give 4b in 82% yield: 'H NMR (DMSO-4,) 8_1.52 (s. 3H, (CH₂),CH) 1.55 (s. 3H, (CH₂),CH), 1.62 (ri, 1 H, J = 8.1 Hz, (CH₃),CH), 2.22 (s. 3H, CH₂Ar), 2.57 (s. 3H, CH₃Ar), 2.97 (s. 2H, CH₂C(=0)), 3.41-4.27 (m, 5 H, nm-C's H-2'-H5'), 6.09 (d. 1H, J = 5.4, ara-C's H-1'), 6.67 (s. 1H, Ar-H), 6.90 (s. 1H, Ar-H), 7.12 (d. J = 5.4, H-6), 8.05 (d. J = 8.1, H-5), 8.67 (bs. 1H, 1TA), "C NMR (DMSO-d.) 8_15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 156.72, 162.02, 169.68,

30 Example 5.

171.87.

Compound 6a. A mixture of PEG-espartic acid (mw. 40,000, 5, 3 g. 0.074 mmol), 4p (385.6 mg, 0.74 mmol), NMM (240 mg, 2.38 mmol), HOBT (120.5 mg, 0.89 mmol), and

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EDC-HCl (228.4 mg, 1.19 mmol) in unhydrous DCM (50 mL) was stirred at 0 °C for 30 minutes. The reaction was allowed to warm to room temperature and continued for 3 days and filtered. The filtrate was concentrated in vacuo and the residue recrystallized from IPA to give 2.7 g (90%) of product. The amount of sra-C in the product measured by UV assay was 2.11 wt%: ¹³C NMR 6 14.40, 19.22, 24.86, 31.17, 38.26, 38.90, 47.94, 48.67, 49.66, 60.17, 61.12, 61.90, 67.96-70.87 (IPEG), 71.70, 74.50, 85.01, 87.53, 95.28, 121.39, 121.18, 132.68, 133.19, 134.77, 137.70, 145.26, 136.93, 155.23, 160.12, 161.56, 168.39, 170.72, 170.92, 171.27, 171.34.

Example 6.

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Compound 6b. Compound 4b was subjected to the same condition as in Example 5 to give 6b in 88% yield. The annum of ara-C in the product measured by UV assay was 1.68 wr%: "C NMCR 6 18.12, 16.22, 24.52, 24.73, 29.55, 30.55, 31.15, 38.04, 38.59, 47.66, 49.16, 49.93, 50.18, 60.93, 61.12, 62.90, 69.44-71.59 (PEG), 71.70, 74.50, 84.78, 54.90, 67.53, 94.85, 127.60, 130.20, 135.51, 136.10, 141.70, 145.15, 147.50, 155.00, 161.20, 169.47, 170.62, 170.92, 171.27.

Example 7.

Compound 9. PEG dial (7, 55 g, 1.38 numol) was azeotroped in tolucne over a 2 hour period followed by removal of 200 mL of solvent by rotary evaporation. The solution was cooled to ~30 "C and triphosgene (0.544 g, 1.83 mmol) was added as solid followed by anhydrous pyridine (0.434 g, 5.49 introl), and the reaction mixture stirred at 50 °C for 1 hour. N-hydroxyptabelimide (8, 1.12 g, 6.88 mmol) and anhydrous pyridine (0.54 g, 6.88 mmol) were added to the chloroformate ribiture and the reaction stirred for a further 2 hours at 50 °C then for 12 hours at room temperature. The reaction mixture was filtered through filter poper and the solvent removed in vacuo and the product crystallized from mentylene chloride-ednyl efter (1100 mL, 8:2, wv) to give the product (50.9 g, 92%): "C NMR & 123.62, 128.10, 134.55, 152.00, 160.00.

Example 8.

PEG-cmc-Asp-O-4-Bu (11). Compound 9 (mw. 40,000, 20 g, 0.459 mmol) and aspartic anid di Abutyl order HCl (18, 1.0 g, 3.55 mmol) were dissolved in anhydrous DCM4, followed by addition of DMAP (0.433 g, 3.55 mmod). The solution was refluxed overnight followed by precipitation by addition of cityl ether (1 L). The solid was isolated by filtration and recrystallized from PA (1 L) twice. The filter cake was washed

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with IPA (200 mL) and other (200 mL) to give 15.6 g (78%) of product after drying at 45 °C in wacur: °C NMR 5 27.837 (CH₂CO₂C(CH₂)₂), 27.991 (CHCO₂C(CH₂)₃), 37.752 (CHCH₂CO₂), 50.800 (NHCH), 64.212 (OCH₂CH₂OC₂O)NII), 81.333 (CH₂CO₂C(CH₂)₃), 82.007 (CHCO₂C(CH₃)), 155.924 (OCH₂CH₂OC(CH₃)), 169.674 (CH₂CO₂C(CH₃)₃), 169.969 (CHCO₂C(CH₃)₃).

Example 9.

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Example 9.

PEG-emr-Asp-OH (12). Compound 11 (15 g. 0.375 mmol) was dissolved in DCM (150 mL) followed by the addition of TFA (75 mL). The solution was stirred at room temperature for 2 hours and hexame (500 mL) added to precipitate the solid. The solid was miturated with hexame to remove TFA followed by recrystallization from chilled DCM-ether. The recrystallized solid was redissolved in DCM (150 mL) and washed with water (150 mL). The organic layer was acparated, dried over unhydrous MgSO₁, concentrated in vacuue, and precipitated with other to give 12.4 g (83%) of product:

"C NMR 5 36.441 (CHCR₂CO₂), 50.177 (NHCH), 64.390 (OCH₂CH₂OC(-O)NH), 81.333 (CH₂CO₂CC(CH₂)), 82.007 (CHCO₂C(CH₂)), 156.172 (OCH₂CH₂OC(-O)NH), 171.944 (CH₂CO₂C(CH₂)), 172.211 (CHCO₂C(CH₂)).

Example 10.

Boc-Asp-Asp-ONic (15). EDC-HCl (2.47 g. 12.86 ramol) was added to a mixture of BockH-aspartic acid (13, 1 g. 4.29 mmol), aspartic acid dimethyl ester-HCl (14, 1.86 g. 9.43 ramol), and DMAP (2.47 g. 12.86 mmol) in anhydrous DCM (30 mL) and DMF (2 ml) at 0 °C. The trixture was allowed to warm up to room temperature overnight. The inixture was washed with 1N HCl three times and the organic layer was dried over anhydrous MgSO₄, followed by removal of the solvent in vacuo to give the product (2.0 g. 90%): "HNMR 8 I.45 (s. 914), 2.62-3.02 (m. 6H, 3 × ClI), 3.70 (s. 6H, 2 × CCI₃), 3.74 (a. 31, CCI₃), 3.75 (s. 3H, CCI₃), 4.50 (b. 1H, CI₃), 4.85 (m. 2H, 2 × CI₃), 6.05 (d. J = 8.05 Hz, 1H, NII), 7.57 (d. J = 7.69 Hz, 1H, NII).

Asp-Asp-4)Me (16). Compound 15 (2.0 g. 3.35 mmol) was dissolved in DCM (30 mL) and TFA (15 mL) and the solution was stirred for 2 h at room temperature. The solvent was removed in vacuo and the residue was receptualized twice with DCM-ether to give the product (1.74 g. 57%) as a white solid: ¹⁰C NMR 8 35.52, 48.76, 50.12, 51.90, 51.96, 52.65, 114.59, 113.49, 168.43, 170.02, 170.92, 171.17, 171.40, 171.48.

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Example 12.

PEG-eme-Asp-Asp-OMe (17). DMAP (4.5 g, 36.86 mroot) was added to a solution of 9 (mw, 40,000, 74 g, 1.84 mroot) and 16 (9.83 g, 18.43 mroot) in 700mL of enhydrous chloroform. The reaction mactive was refluxed for 24 hours under nitrogen. The reaction was cooled to room temperature and concentrated to ¼ volume. Crude product was precipitated with 2.5 L of ether, filtered and recrystallized from 5.5 L of PA (65°C). The product was filtered and washed twice with fresh IPA, twice with fresh ether, and dried overnight at 40 °C to yield 59.0g (84%) of 17: °C NMR & 35.344, 36.931, 48.082, 48.208, 50.835, 51.509, 52.239, 61.045, 63.953, 68.854-72.056, 155.538, 170.102.

Example 13.

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PEG-eme-Asp-Asp-OH (18). Compound 17 (51 g. 1.26 mmol) and LiOH-H₂O (0.8 g. 18.9 mmol) were dissolved in 300 mL of water and the solution stirred overnight at rown temperature. The pH of the solution was adjusted to 2.5 by the addition of 1N HCl. The solution was extracted with DCM (3 × 600 mL), the organic layers combined, dried over analysinous MgSO₄ and concentrated in sectio. The residue was recrystallized from DCM-ether to give the product which was collected by fifts aton and dried at 40 °C overnight to yield 38 g (54%) of the octa-acid: ¹¹C NMR (D₂O) § 38.384, 39.704, 51.951, 54.465, 62.934, 67.105, 71.445-74.381 (FBG), 159.772, 173.831, 174.940, 176.359, 176.696.

20 Example 14.

Mei-OMe (20). Melphalan (19, 1.00 g. 3.2kmmol) was suspended in 2,2 dimethoxy-propme (65.59 mL, 533.49 mmol). To the suspension was added aqueous HCi (36 %, 5.28 mL) and absolute methanol (4 mL). The mixture was warmed to mild reflux with vigorous sturing until solution started to turn slightly brown, followed by stirring at room temperature for 18 hours. The reaction mixture was concentrated in vacuo and the crude product precipitated from the residue with other. The solid was filtered, washed with ether, and purified by silico gel column chromatography (CilCi₁: McOH = 9:1, wh) to yield the desired product (0.47 g. 4%): "C NMR 8 39.751, 40.340, 51.912, 53.435, 55.803, 112.124, 126.076, 130.620, 145.033, 175.754.

30 Example 15.

Hoo TML1p-Met-OMe (22). EDC (0.52 g. 2.70 mmsl) and DMAP (0.988 g. 8.10 mmsl) were added to a mixture of 21 (0.531 g. 1.35 mmsl) and 20 (0.863 g. 2,70 mmsl) in

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aninydrous DCM (15 mL) and anhydrous DMF (5 mL) at 0 °C in an ice bath. The renotion mixture was stirred at room temperature overright under nitrogen then concentrated in vocuo. The residue was redissolved in DCM (75 mL) and washed three times with 25 mL 1N HCl. The originic hyer was dried over anhydrous magnesium sulfate, concentrated, and purified by silica gal column chromatography (cfuy) actual:heanne ~ 7:3, v/v) to yield the desired product (0.757 g. 80.8 %): "C NMR & 20.120, 25.306, 28.294, 31.768, 35.427, 35.947, 36.669, 39.505, 40.311, 49.324, 51.959, 52.234, 53.453, 79.467, 112.095, 123.374, 125.169, 130.439, 132.856, 133.427, 136.666, 138.697, 145.091, 149.841, 156.081, 170.888, 172.298.

10 Example 16.

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TMLIB-Mel-OMe TFA Salt (23). Compound 22 (0.757 g. 1.09 mmol) was stirred in DCM (5mL) and TFA (2.5 mL) at room temperature for 2 hours. The reaction subution was concentrated, redissolved in minimal DCM, and precipitated with other. The product was collected by filtration to yield the desired product (0.222g. 35.9 %): "C NMR (CDCI, ÷ CD,OD) 8 20.026, 25.146, 31.738, 31.892, 35.271, 36.219, 39.163, 40.340, 49.006, 52.219, 53.396, 112.073, 123.260, 124.756, 130.377, 133.026, 133.180, 136.815, 138.595, 145.110, 149.283, 171.069, 171.619, 172.630.

Example 17.

PEG-cine-TMLIJ-Mei-OMe (24). A mixture of PEG-cine-Asp-Asp-OH (12, 1.6g, 0.039 lmmol), 23 (0.277g, 0.391 mmol), EDC (0.076g, 0.391 mmol), and DMAP (0.155g, 1.269 mmol) in mbydrous DCM (23 mL) and enhydrous DMF (6 mL) was stirred overnight at room temperature under nitrogen. The solution was concentrated in vacuo and the residue recrystallized from 130 mJ. IPA to yield the product (1.543g, 92.5 %). The amount of melphalan in the product measured by UV assay was 2.86% wd/w: "CNMR 8 19.642, 24.788, 31.175, 34.350, 35.975, 38.817, 39.905, 48.558, 51.553, 52.803, 60.897, 62.331, 65.145-72.878 (PEG), 111.394, 122.761, 124.425, 129.698, 132.105, 132.878, 135.804, 137.737, 144.316, 149.065, 160.432, 170.608, 171.598.

Example 18.

Roe-TML1B-AraC (25). A solution of Ara-C (1, 9.88 g, 40.66 mmol) in anhydrous pyridine (85 mL) was added to a mixture of 21 (4.0 g, 10.17 mmol), HOST (5.49 g, 40.66 mmol), EDC (15.61 g, 81.32 mmol), and NoMM (8.93ml, 8.21g, 81.32mmol, 8eq) in anhydrous pyridine (200 mL). The reaction mixture was stored for 48 hours at 40 °C

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under nitrogen, followed by concentration in same. The residue was redissolved in DCM (300 mL), washed three times with water (100 mL) and twice with 0.1N HCl (100 mL). The organic layer was dried over magnesium sufface, concentrated, and parified by silica gel column chromatography (C3Cl₃ · MEDH = 9.1, 4/v) to yield the desired product (3.26 g., 52 %): "C NMR 8 20.315, 25.560, 28.512, 31.660, 33.520, 36.200, 39.221, 50.239, 61.719, 75.171, 76.698, 79.635, \$5.341, 88.052, 96.435, 122.894, 132.519, 133.190, 136.166, 138.007, 146.222, 149.109, 155.906, 162.191, 171.733. Example 19.

TML1β-AruC TFA salt (26). Compound 25 (3 g, 4.85 mmol) was dissolved in DCM (15 mL) followed by addition of TFA (7.5 mL) at 0 °C. Reaction aristure was stirred at 0 °C for 1.2 hours and concentrated in vacuo in a cool water bath. Residue was precipitated with DCM-ether to yield 0π desire product (2.37 g, 77 %): ¹²C NMR (CDCl₃ + CD₃OD) δ 20.0, 25.3, 31.5, 31.7, 35.0, 38.9, 50.2, 60.9, 75.1, 75.8, 85.7, 88.1, 94.9, 109.7, 113.5, 117.3, 121.1, 122.5, 132.6, 136.4, 138.4, 148.7, 149.5, 150.1, 159.2, 159.6, 160.1, 160.6, 161.1, 170.6, 172.7

Example 20.

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PEG-cmc-Asp-Asp-TML1B-AraC, octamer (27). Compounds 26 and 18 were subjected to the same condition as in Example 18 to prepare 27.

Example 21.

20 In vitro and in vivo data for compounds 6a and 6b.

in this Example, in vivo and in vitro data are presented and compared to termodified Are-C.

in Vivo

Athymic mude mice were implanted subcuraneous with a 4-5 mm² tissue fragment of LX-1 enflected from donor mice. The turnor crocar site was observed twice weekly and measured once palpable. The turnor volume for each measure was determined by measuring two dimensions with calipers and calculated using the formula: turnor volume ~ (length x width²)/2. When turnors received the average volume of 90 mm², the mice were divided into their experimental groups which consisted of unmodified Ara-C and PEG-Ara-C compounds. The mice were sorted to evenly distribute turnor size, grouped into 4 to 6 mice/group, and car purched for permanent identification. Drugs were ediministered instravenously q3d x 4 (Dzy 1, 4.7 and 10) via the tail vein at an approximate rate of 0.5

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ml. per minute. Compounds were given both at an equal molar basis (absolute smount of sotive) of 20 mg/kg and at close their respective MTO (Ars-C, 100 mg/kg/dose (toxicity); 6a and 6b, 40 mg/kg/dose (volume). Mouse weight and tumer size were measured at the beginning of study and twice weekly through week 4. Drug effectiveness was determined by comparing turnor growth in treated versus untreated (no wehicle) control mice. Five types of codpoints were used as the basis for comparison: (a) mean tumor volumes at Day 28; (b) mean percent change in individual turnor volumes from initial; (c) percent difference in tumor volume (%T/C), measured when the control group's median tumor volume reached approximately 800 - 1100 rom³ (exponential growth phase); (d) percent difference in tumor volume (%T/C) at Day 21 (~2000 mm³) and (e) the number of tumor regression (smaller tumor volume on Day 28 compared to Day 1) per group.

Compound 6b demonstrated better antitumer activity than native Ara-C at only 20% of the active parent compound's dose. Compound on also demonstrated significant efficacy. Although the %T/C was about twice of that which was recorded for 6b, it nonetheless compared vary favorably against native Ara-C, especially considering that the inventive compound was given at only 20% of the active parent compound's dose.

Cempound	I _{1/2} (h)* Rat Plusma	IC ₈₀ (nM)* P388/U	LX-1 %T/C*
Ara-C		10	74.0 (100 mg/kg)
Compound (a.	2.1	123	122 (20 mg / kg)
Сипроилд бр	53	958	59.3 (20 mg/kg)

" All experiments were done at 37 °C in duplicate and t_{L0} was measured by the disappearance of PEG derivatives. Standard deviation of measurements = 110 %. "Moun baseline autor volume was 1000 mm".

IN VITRO BLOASSAY

A series of in vitre assays were conducted to determine the IC, so for unmodified Ara-C and compound 10 using the P388/O (murine lymphoid neoplasm, Southern Research Institute) cell line. The P388/O cells were grown in RPMI 1640 medium (Whittaker Bioproducts, Walkersville, Maryland) + 1074 FH8 (Hyclone Inc., Logan 171). Binessays were performed in their respective media commining audibiotics and fungizone.

12°C) 82°C6606A

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Ara-C was dissolved in DMSO and diluted to the appropriate concentration in culture media. The PEG-Ara-C compounds were dissolved in water and diluted to the appropriate concentrations in culture media.

The assays were performed in duplicate in 96-well microtiter cell culture plates. Two fold serial dilution of the compounds were done in the uncrotiter plates. Cells were detached by incubeting with 0.1% Trypsin/Versone is 37°. Trypsin was inactivated by adding the appropriate media for each cell line containing 10% FBS. To each well of the microtiture plates, 10,000 cells were added. After three days, cell growth was measured by addition of a metabolic indicator dye, Alamar Blue, according to the manufacturer's protocol. The IC₁₅ value for the test compounds and reference compound are provided above in the Table.

While there have been described what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that changes and modifications may be made without departing from the spirit of the invention. It is intended to claim all such changes and modifications as fall within the true scope of the invention.

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WHAT IS CLAIMED IS:

1. A compound comprising the formula:

(f)

wherein: R_1 is a polymeric residue; Y_1 is O, S or NR_2 ; E_1 is R_2 R_3 R_4 R_3 R_4 R_3 R_4 R_5 R_5 R_5 R_5 R_5 R_5 R_5 R_5 R_6 R_7 R_7

- (a) is sero or one;
- (m) is zero or a positive integer;
- (n) and (p) are independently 0 or a positive integer;
- Y_{2a} are independently O, S or NR₁₀;

 $R_{\lambda,a}$ are independently schemed from the group consisting of hydrogen, $C_{1,a}$ alkyls, $C_{\lambda,a}$ branched alkyls, $C_{\lambda,a}$ cycloalkyls, $C_{1,a}$ substituted alkyls, $C_{\lambda,a}$ substituted cycloalkyls, aryls, substituted aryls, arabkyls, $C_{1,a}$ alkoxy, phenoxy and $C_{1,a}$ heteroalkyls, $C_{1,a}$ alkoxy, phenoxy and $C_{1,a}$ heteroalkoxy;

D, and D, are independently OH.

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$$-j - \left\{ \begin{array}{c} 1 \\ 1 \end{array} \right\} \left\{ \begin{array}{c} 1 \end{array} \right\} \left\{ \begin{array}{c} 1 \\ 1 \end{array} \right\} \left\{ \begin{array}{c} 1 \end{array} \right\} \left\{ \begin{array}{c$$

or a terminal branching group;

wherein (v) and (t) are independently 0 or a positive integer up to about 6;

L; and L; are independently selected bifurctional linkers;

heteroalkyla, C_{1a} aikmy, phenoxy and C_{1a} heteroaloxy: Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocurbon or a multi-substituted heteroxyclic group;

 R_1 and R_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing moieties.

The compound of claim 1, wherein R, further comprises a capping group A, selected from the group consisting of hydrogea, NH₁, OH, CO₂H, C₁₄ moieties and

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$$E_{2} = \begin{bmatrix} E_{1} & Y_{1} & \\ \vdots & \vdots & \vdots \\ E_{3} & E_{n} & \end{bmatrix}$$

3 A compound of claim 2, comprising the furnalla:

$$E_{2} = \begin{bmatrix} E_{1} & Y_{1} \\ \vdots & \vdots \\ E_{3} & E_{4} \end{bmatrix} \times \begin{bmatrix} M \\ \alpha \end{bmatrix}_{a} \begin{bmatrix} R_{2} \\ \vdots \\ R_{3} \end{bmatrix}_{m} \times \begin{bmatrix} R_{2} \\ \vdots \\ R_{6} \end{bmatrix}_{m} \times \begin{bmatrix} M \\ \alpha \end{bmatrix}_{a} \begin{bmatrix} M \\ \vdots \\ E_{4} \end{bmatrix}_{E_{3}} = E_{2}$$

4. The compound of claim 1, wherein said terminal branching group comprises the

uherei-

u is $\frac{\left(\begin{array}{c} R_{v} \\ C \end{array} \right) \left(\begin{array}{c} C \\ D \end{array} \right)}{n}$

 $E_{\rm phys}$ are independently H, $E_{\rm ts}$ or



(n) and (p) are independently 0 or a positive integer,

Y₂₋₃ are independently O, S or NR₁₀;

 R_{d+b} are independently selected from the group consisting of hydrogen, $C_{1:d}$ alkyls, $C_{1:d}$ branched alkyls, $C_{1:d}$ cyclosikyls, $C_{1:d}$ substituted alkyls, $C_{1:d}$ substituted cyclosikyls, aryls, substituted aryls, araikyls, $C_{1:d}$ heteroalkyls, substituted $C_{1:d}$ heteroalkyls, substituted $C_{1:d}$ heteroalkyls, substituted $C_{1:d}$ heteroalkyls, substituted $C_{1:d}$

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alkyle, C_{14} alkoxy, phenoxy and C_{14} heteroalkney: D'_1 and D'_2 are independently OH,

wherein (v) and (i) are independently 0 or a positive integer up to about δ_i

 \mathbf{L}_1 and \mathbf{L}_2 are independently selected bifunctional linkers;

 $Y_{\bullet,i}$ are independently selected from the group consisting of Q_i , S and $NR_{i,i}$, $R_{i+1,i}$ are independently selected from the group consisting of hydrogen,

 $C_{1,a}$ alkyls, $C_{1,a}$ transched efkyls, $C_{1,a}$ cyclosalkyls, $C_{1,c}$ substituted alkyls, $C_{2,a}$ substituted cyclosalkyls, aryls, substituted aryls, aralkyls, $C_{1,a}$ beternalkyls, substituted $C_{1,c}$ beternalkyls, $C_{1,a}$ alkovy, phenoxy and $C_{1,c}$ internalkyls, $C_{1,c}$ internalkyls, $C_{1,c}$ internalkyls, $C_{1,c}$ internalkyls, $C_{1,c}$ internalkyls, $C_{1,c}$ internal $C_{1,c}$ internalkyls, $C_{1,c}$ in

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At its a molety which when included in Formula (I) forms a multi-substituted aromatic hydrocurbon or a multi-substituted heterocyclic group;

B, and B, are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing maieties;

 $E_{45-\epsilon_0}$ are independently II, E_{41} or

wherein

D'', and D''2 are independently OH,

œ

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- The compound of claim 3, Y₁ is O.
- The compound of claim 1, wherein R_i comprises a polyalkylene axide residue.
- The compound of claim 6, wherein R₁ comprises a polyethylene glycol residue.
- 8. The compound of claim 3, wherein $R_{\rm f}$ comprises a polyethytene glycol residue.
- 9. The compound of claim 6, wherein R; is selected from the group consisting of
 -C(=Y_0)-C(H_0)-O-(CH_0CH_0)_v.A.
 -C(=Y_0)-Y_1-(CH_0)-O-(CH_0CH_0)_v.A.
 -C(=Y_0)-NR_{11}-(CH_0)-O-(CH_0CH_0)_v.A.
 -(CR_0,R_1)-O-(CH_0)-O-(CH_0CH_0)_v.A.
 -NR_{10}-(CH_0)-O-(CH_0CH_0)_v.A.
 -(CY_0)-C(H_0)-O-(CH_0CH_0)_v.A.
 -(C(-Y_0)-Y_1-(CH_0)-C(CH_0CH_0)_v.A.
 -(C(-Y_0)-Y_1-(CH_0)-C(CH_0CH_0)_v.A.
 -(C(-Y_0)-Y_1-(CH_0)-C(CH_0CH_0)_v.A.
 -(CR_0,R_{11})-O-(CH_0CH_0)_v.A.
 -(CR_0,R_{11})-O-(CH_0CH_0)_v.A.
 -(CR_0,R_{11})-O-(CH_0CH_0)_v.A.
 -(CR_0,R_{11})-O-(CH_0CH_0)_v.A.
 -(CR_0,R_{11})-O-(CH_0CH_0)_v.A.

 -NR_{11}-(CH_0,R_0)-(CH_0CH_0)_v.A.
 -(NR_0,CH_0,R_0)-(CR_0,R_0).
 -(NR_0,CH_0,R_0)-(CH_0,R_0).
 -(NR_0,CH_0,R_0)-(CH_0,R_0).
 -(NR_0,CH_0,R_0)-(CH_0,R_0).
 -(NR_0,CH_0,R_0)-(CH_0,R_0).
 -(NR_0,R_0)-(CH_0,R_0).
 -(NR_0,R_0)-(CH_0,R_0).
 -(NR_0,R_0)-(CR_0,R_0).
 -(NR_0,R_0)-
- R_{2a} , R_{2a} and R_{2a} are independently selected from among H, C_{1a} alkyls, $C_{h,ij}$ branched alkyls, C_{2a} cyclealkyls, C_{1a} substituted alkyls, C_{2a} substituted cyclealkyls, aryls, substituted cryls, aralkyls, C_{1a} alkery, phenuxy and C_{1a} beteroalkyls, substituted C_{1a} heteroalkyls, C_{1a} alkery, phenuxy and C_{1a} beteroalkexy;
 - c and f are independently zero, one or two; and A is a capping group.
- 10. The compound of claim 9, wherem R_1 comprises -O-(CH₂CH₂O), and x is a positive integer so that the weight average molecular weight is at least about 20,000.

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- 11. The compound of claim 3, wherein $R_{\rm c}$ has a weight average molecular weight of from about 20,000 to about 100,000.
- 12. The compound of claim 3, wherein R_t has a weight average molecular weight of from about 25,000 to about 60,000.
- 13. A compound of claim 3, comprising the formula

$$\begin{array}{c} C_1 \\ C_2 \\ C_3 \\ C_4 \\ C_5 \\ C_6 \\ C_7 \\ C_8 \\$$

14. The compound of claim 13, wherein D₁ is

12°C 62.*O6606A

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15. The compound of claim 13, wherein D₁ is

- 16. The compound of claim 1, wherein L_1 is (CH₂CH₂O)₂.
- 17. The compound of claim 1, wherein L_2 is selected from the group consisting of $-CH_2$, $-CH_1(CH_2)$, $-CH_2(CO)NHCH(CH_3)$, $-(CH_2)$, $-CH_2(CO)NHCH_2$, $-(CH_2)$, -NH, $-(CH_2)$, $-(CH_2)$, -
- 18. A compound of claim 1, selected from the group consisting of:

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wherein $R_{\rm c}$ is a PRG residue and D is selected from the group consisting of:

where \boldsymbol{B} is a residue of an armine or a hydroxyl- containing drug.

- 19. A computed of claim 18, wherein B is a residue of a member of the group consisting of: datmorphicin, doxombicin; p-eminoemiline mustard, melphalan, Arn-C (cytosine arabinoside), lencinc-Ara-C, and generalishine
- 20. A method of treatment, comparising administering to a mammal in need of such treatment an effective amount of a compound of claim 1, wherein D, is a residue of a biologically active moiety.
- A method of treatment, comprising administering to a maximal in need of such treatment an effective amount of a compound of claim 18.

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22. The compound of claim 1, wherein Ar comprises the formula:

wherein R_{i1} and $R_{i2,20}$ are individually selected from the group consisting of hydrogen, C_{14} alkyls, C_{12} branched alkyls, C_{34} evolotikyls, C_{1} , substituted oyeloolkyls, aryls, substituted oyeloolkyls, aryls, substituted aryls, aralkyls, C_{14} beteroolkyls, substituted C_{14} beteroolkyls, C_{14} alkoxy, phenoxy and C_{14} beteroakoxy.

- 23. The compound of claim 22, wherein $R_{\rm G}$ and $R_{\rm chis}$ are each H or CH $_{\rm 3}$
- 24. A method of preparing a prolymer conjugate, comprising: reacting a compound of the formula (VIII):

wherein

(v) and (i) are independently 0 or a positive integer up to about 6;

48

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 \mathbf{L}_1 and \mathbf{L}_2 are independently selected bifunctional linkers;

 $Y_{\rm e,j}$ are independently selected from the group consisting of O, S and NR $_{\rm rr}$

Report are independently selected from the group consisting of hydrogen,

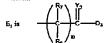
 C_{14} alkyls, $C_{3\cdot 2}$ branched alkyls, C_{34} eyeloslkyls, C_{14} substituted alkyls, C_{14} substituted cyclonikyls, aryls, substituted aryls, aralkyls, C_{14} beteroalkyls, substituted C_{14} beteroalkyls, C_{14} alkvay, phenoxy and C_{14} beteroalkoxy;

Ar is a moirty which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted beterocyclic group; and

 B^2 , is a residue of a hydroxyl- or an amine-containing moiety; with a comprand of the formula (DN):

$$R_1 = \begin{cases} R_2 \\ R_3 \\ R_3 \end{cases} m \begin{cases} M_1 \\ R_2 \\ R_3 \end{cases} = \begin{cases} R_2 \\ R_3 \\ R_3 \end{cases} = \begin{cases} R_3 \\ R_3 \\ R_3 \\ R_3 \end{cases} = \begin{cases} R_3 \\ R_3 \\ R_3 \\ R_3 \\ R_3 \end{cases} = \begin{cases} R_3 \\ R_3 \\$$

wherei



 $E_{n,q}$ are independently H, E_{σ} or



 D_3 and D_4 are independently OH, a leaving group which is capable of reacting with an unprotected scales or bydroxyl or a terminal branching group;

R, is a polymeric residue;

Y, is O, S or NR,

M ts O, S or NR,;

(a) is zero or one;

(m) is 0 or a positive integer;

(n) and (p) are independently 0 or a positive integer;

 $Y_{a,a}$ are independently O. S or NR_{aa} and

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 $R_{_{1:0}}$ are independently selected from the group consisting of hydrogen, $C_{1:4}$ alkyls, $C_{1:2}$ branched alkyls, $C_{1:4}$ cycloalkyls, $C_{1:4}$ substituted cycloalkyls, cryls, substituted cycloalkyls, cryls, substituted cycloalkyls, cryls, substituted cycloalkyls, cryls, substituted $C_{1:4}$ heteroalkyls, $C_{1:4}$ betteroalkyls, $C_{1:4}$ betteroalkyls, $C_{1:4}$ betteroalkyls, cycloalkyls, $C_{1:4}$ betteroalkoxy; under conditions sufficient to cause a polymeric conjugate to be formed.

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Fig. 1

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Fig. 2

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【国際調査報告】

	INTERNATIONAL SEARCH REPO	ROT .	International sp FCT/USes/or		
A. CLASSFICATION OF SUBJECT MATTER IPO(1) AN IS 17/50, 20/564, 20/54 US CL. "Please for Extra Nicet. According to International Parism Classification (IPC) or so both engines described and IPC D. FIELDS SEARCHED					
Minimum documentation searched (classification system followed by chasification symbols)					
U.S. : 500/302, 403, 403, 203/54.1; 560/189, 179, 200, 514/779.0. 515, 513, 615, 426/193.1, 1931, 170.1					
Documentation searched other than minimum decommendation to the extent that such documents are anduded in the fields ANGINE.					
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C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category*	Citation of document, with indication, where a	ppropriate, of the relev	ant pastages	Relovant to claim No.	
У	GREENWALD et al.; Drug delivery systems based on trimethyl lock lactonization: Poly(ethylene glycol) Products of amino-containing compounds; 2000, Chem Abstract 132: 227266				
Υ	GREENWALD et al; "TriaRyl-lock-facilitated polymeric prodrugs of amino-containing bioactive agents"; 1999; Chem Abstract 131; 92540				
Vurther documents are fisted in the continuation of Box C. See patent family unorx.					
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A. CLASSIFICATION OF SUBJECT MATTER: US CL.					
322/337, 401, 923; 323/54.1; SAN/303, 179, 590, 514/772.3, 615, 616; 454/194.1, 194.1, 178.1					
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(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZM,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),OA(BF,B),CF,CG,CI,CM,GA,GN,GQ,QW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,OM,PH,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TN,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZM,ZW

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> 4J005 AA02 BD05 BD06 4J031 CD13

【公報種別】特許法第17条の2の規定による補正の掲載 【部門区分】第3部門第3区分

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C 0 8 G 85/00

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【手続補正書】

【提出日】平成16年9月29日(2004.9.29)

【手続補正1】

【補正対象書類名】特許請求の範囲

【補正対象項目名】全文

【補正方法】変更

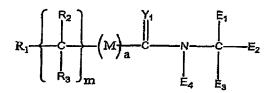
【補正の内容】

【特許請求の範囲】

【請求項1】

【载:1】

(I)



|式中、

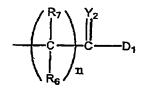
R₄は高分子残基であり;

Y, はO、SまたはNR。であり;

MはO、SまたはNR、であり;

E, は

【化2】



であり;

Ez-4は独立に、H、E、または

[化3]

であり;

(a)は0または1であり;

(m)は0または正の整数であり;

(n)および(p)は独立に、0または正の整数であり;

Y,_,は独立に、O、SまたはNR,oであり;

 R_{-10} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{4-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} へテロアルキル、置換 C_{1-6} へテロアルキル、置換 C_{1-6} へテロアルコキシからなる群から選択され;

D₁およびD₂は独立に、OH、

【化4】

(式中、

(v)および(t)は独立に、0または約6までの正の整数であり; JはNR₁₂または 【化5】



であり;

L,およびL,は独立に選択された二官能性リンカーであり;

Y₄₋₇は独立に、O、SおよびNR₁₄からなる群から選択され;

 $R_{1_{1-1_4}}$ は独立に、水素、 G_{-6} アルキル、 G_{-1_2} 分枝鎖アルキル、 G_{-8} シクロアルキル、 G_{-6} 置換アルキル、 G_{-6} 置換シクロアルキル、アリール、置換アリール、アラルキル、 G_{-6} でファルキル、 置換 G_{-6} でファルキル、 G_{-6} アルコキシ、フェノキシおよび G_{-6} でロアルコキシからなる群から選択され:

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり;

B₁およびB₂は独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含有成分の 残基からなる群から選択される)

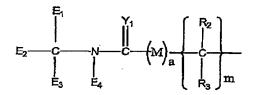
または末端分枝基であると

で表される化合物。

【請求項2】

R₁が水素、NH₂、OH、CO₂H、C₁₋₆基および

[化6]



からなる群から選択されるキャッピング基Aをさらに含んでなる、請求項1に記載の化合物。

【請求項3】

式:

【化7】

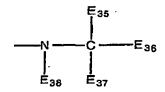
$$E_{2} = \begin{bmatrix} E_{1} & Y_{1} & E_{1} \\ \vdots & \vdots & \vdots \\ E_{3} & E_{4} & E_{4} \end{bmatrix} \begin{bmatrix} R_{2} & R_{1} & R_{2} \\ \vdots & \vdots & \vdots \\ R_{3} & M & E_{4} \end{bmatrix} \begin{bmatrix} R_{2} & R_{1} & R_{2} \\ \vdots & \vdots & \vdots \\ R_{3} & M & E_{4} & E_{3} \end{bmatrix} E_{2}$$

で表される、請求項2に記載の化合物。

【請求項4】

上記末端分枝基が式:

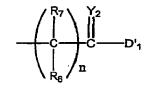
【化8】



【式中、

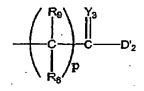
E3, it

【化9】



であり;

E₃₆₋₃₈は独立に、H、E₃,または 【化 1 0】



であり;

(n)および(p)は独立に、0または正の整数であり;

Y₂₋₃は独立に、O、SまたはNR₁₀であり;

 R_{6-10} は独立に、水素、 G_{-6} アルキル、 G_{5-12} 分枝鎖アルキル、 G_{5-8} シクロアルキル、 G_{5-6} 置換アルキル、 G_{5-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 G_{5-6} へテロアルキル、置換 G_{5-6} へテロアルキル、 G_{5-6} アルコキシからなる群から選択され;

D',およびD',は独立に、OH、

【化11】

または 【化12】

|式中、

(v)および(t)は独立に、Oまたは約6までの正の整数であり;

LaおよびLaは独立に選択された二官能性リンカーであり;

Y₄₋₇は独立に、O、SおよびNR₁₄からなる群から選択され;

 R_{11-14} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{1-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} へテロアルキル、置換 C_{1-6} へテロアルキル、 C_{1-6} アルコキシ、フェノキシおよび C_{1-6} へテロアルコキシからなる群から選択され;

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり;

B、およびB、は独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含有成分の 残基からなる群から選択され;

E45 は

$$\begin{array}{c|c}
 & \text{Alt 1 3} \\
 & \text{C} \\
 & \text{C} \\
 & \text{C}
\end{array}$$

であり;

E₄₆₋₄₈は独立に、H、E₄,または 【化14】

、中为

$$-J - \left\{L_{1}\right\}_{V} \left\{L_{2}\right\}_{t} \left\{L_{2}\right\}_{t} \left\{L_{2}\right\}_{t} \left\{L_{2}\right\}_{t} \left\{L_{3}\right\}_{t} \left\{L_{4}\right\}_{t} \left\{L_{5}\right\}_{t} \left\{L_{5}\right\}$$

または 【化16】

である)

である

である]

で表される、請求項1に記載の化合物。

【請求項5】

Y,がOである、請求項3に記載の化合物。

【請求項6】

Rがポリアルキレンオキシド残基を含んでなる、請求項1に記載の化合物。

【請求項7】

R₁がポリエチレングリコール残基を含んでなる、請求項6に記載の化合物。

【請求項8】

R₁がポリエチレングリコール残基を含んでなる、請求項3に記載の化合物。

【請求項 9】 凡が

 $-C(=Y_6)-(CH_3)_6-O-(CH_3CH_3O)_5-A_5$

 $-C(=Y_6)-Y_7-(CH_7)_6-O-(CH_7CH_7O)_2-A_2$

 $-C(=Y_{k})-NR$, $_{3}-(Ol_{2})_{t}-O-(Ol_{3}Ol_{2}O)_{x}-A$

 $-(CR_{24}R_{25})_{e}-O-(CH_{1})_{e}-O-(CH_{1}CH_{2}O)_{e}-A_{1}$

 $-NR_{2,3}$ -(CH₂)_f -0-(CH, CH, 0)_x -A_x

-C(=Y₆)-(CH₂)₁-0-(CH₂CH₂0)₂-(CH₃)₁-C(=Y₆)-(CH₂CH₂0)₂-(CH₃0)₃-(CH₃0)₄-C(=Y₆0)-(CH₂CH₂0)₄-(CH₃0)₄-C(=Y₆0)-(CH₃CH₂0)₄-(CH₃0)₄-C(=Y₆0)-(CH₃CH₂0)₄-(CH₃0)₄-C(=Y₆0)-(CH₃CH₂0)₄-(CH₃0)₄-C(=Y₆0)-(CH₃CH₂0)₄-(CH₃0)₄-C(=Y₆0)-(CH₃CH₂0)₄-(CH₃0

 $-C(=Y_6)-Y_7-(CH_2)_7-0-(CH_3)_7-(CH_3)_7-(CH_3)_7-(CH_3)_7-(CH_3)_7$

 $-C(=Y_6)-NR_2$, $-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_2$, $-C(=Y_6)-(CH_2CH_2O)_x$

 $-(CR_{24}R_{25})_{e}-O-(CH_{20})_{f}-O-(CH_{20})_{x}-(CH_{20})_{f}-O-(CR_{24}R_{25})_{e}-$

 $-NR_{2,3}$ $-(CH_{2})_{f}$ $-0-(CH_{2}CH_{2}0)_{x}$ $-(CH_{2})_{f}$ $-NR_{2,3}$ $-(CH_{2})_{f}$

Y。およびY,は独立に、O、SまたはNR、1,であり;

×は重合度であり;

 R_{23} 、 R_{24} および R_{25} は独立に、H、 G_{-6} アルキル、 G_{-12} 分枝鎖アルキル、 G_{-8} シクロアルキル、 G_{-6} 置換アルキル、 G_{-6} 置換シクロアルキル、アリール、置換アリール、アラルキル、 G_{-6} へテロアルキル、置換 G_{-6} へテロアルキル、 G_{-6} アルコキシ、フェノキシおよび G_{-6} へテロアルコキシからなる群から選択され;

eおよびfは独立に、0、1、または2であり; かつ

Aはキャッピング基である)

からなる群から選択される、請求項6に記載の化合物。

【請求項10】

 R_1 が-O-(OH, OH, O)_xを含んでなり、かつxは重量平均分子量が少なくとも約20,000であるような正の整数である、請求項9に記載の化合物。

【請求項11】

R, の重量平均分子量が約20,000~約100,000である、請求項3に記載の化合物。

【請求項12】

R₁の重量平均分子量が約25,000~約60,000である、請求項3に記載の化合物。 【請求項13】

式

【化17】

で表される、請求項3に記載の化合物。

【請求項14】

D₁が

【化18】

である、請求項13に記載の化合物。

【請求項15】

D₁が

【化19】

である、請求項13に記載の化合物。

【請求項16】

L1が(CH, CH, O),である、請求項1に記載の化合物。

【請求項17】

【請求項18】

【化20】

および 【化21】

|式中、 R₃はPEG残基であり、かつDは

[1L22]

および 【化23】

(式中、

Bはアミンまたはヒドロキシル基含有薬物の残基である) からなる群から選択される(

からなる群から選択される、請求項1に記載の化合物。

【請求項19】

Bがダウノルビシン、ドキソルビシン; p-アミノアニリンマスタード、メルファラン、A ra-C(シトシンアラビノシド)、ロイシン-Ara-C、およびゲムシタビンからなる群のメンバーの残基である、請求項18に記載の化合物。

【請求項20】

治療が必要な哺乳類に投与するための、有効量の請求項1に記載の化合物(式中、D₁は生物学上活性な成分の残基である)を含む医薬組成物。

【請求項21】

治療が必要な哺乳類に投与するための、有効量の請求項18に記載の化合物を含む医薬 組成物。

【請求項22】

Arが式:

【化24】

(式中、

 R_{11} および R_{18-20} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{1-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} ヘテロアルキル、置換 C_{1-6} ヘテロアルコキシからなる群から選択される)

で表される、請求項1に記載の化合物。

【請求項23】

 R_{11} および R_{18-20} が各々、Hまたは CH_{3} である、請求項22に記載の化合物。

【請求項24】

高分子複合体の製造方法であって、

式(VIII):

【化25】

(v)および(t)は独立に、0または約6までの正の整数であり;

JはNR,,または

【化26】

であり;

L,およびL,は独立に選択された二官能性リンカーであり;

 Y_{4-5} は独立に、O、Sおよび NR_{17} からなる群から選択され;

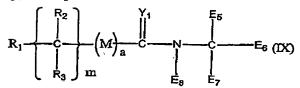
 $R_{1_{-17}}$ は独立に、水素、 $C_{1_{-6}}$ アルキル、 $C_{3_{-12}}$ 分枝鎖アルキル、 $C_{3_{-8}}$ シクロアルキル、 $C_{1_{-6}}$ 置換アルキル、 $C_{3_{-8}}$ 置換シクロアルキル、アリール、置換アリール、アラルキル、 $C_{1_{-6}}$ へテロアルキル、置換 $C_{1_{-6}}$ へテロアルキル、 $C_{1_{-6}}$ でアルコキシからなる群から選択され;

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成

分であり;かつ

 B'_1 はヒドロキシルまたはアミン基含有成分の残基である)で表される化合物と、式(IX):

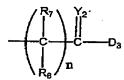
【化27】



(式中、

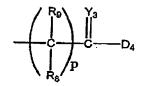
E, 1t

【化28】



であり;

E₆₋₈は独立に、H、E₅または 【化 2 9】



であり:

D,およびD,は独立に、OH、保護されていないアミンまたはヒドロキシルと反応しうる脱離基、または末端分枝基であり;

R₄は高分子残基であり;

Yı はO、SまたはNR。であり、

MはO、SまたはNR₅であり;

- (a)は0または1であり;
- (m)は0または正の整数であり;
- (n)および(p)は独立に、0または正の整数であり;

Y₂₋,は独立に、O、SまたはNR₁,であり;かつ

 R_{2-10} は独立に、水素、 C_{1-6} アルキル、 C_{3-12} 分枝鎖アルキル、 C_{3-8} シクロアルキル、 C_{4-6} 置換アルキル、 C_{3-8} 置換シクロアルキル、アリール、置換アリール、アラルキル、 C_{1-6} へテロアルキル、置換 C_{1-6} へテロアルキル、 C_{1-6} アルコキシ、フェノキシおよび C_{1-6} へテロアルコキシからなる群から選択される)

で表される化合物とを、高分子複合体を生成させるのに十分な条件下で反応させることを含んでなる、上記方法。